

THE EMBRYOLOGICAL DEVELOPMENT
OF THE QUEENSLAND LUNGFISH,
NEOCERATODUS FORSTERI, (KREFFT)

A. KEMP
Queensland Museum

ABSTRACT

The development of *Neoceratodus forsteri* is described for a comparison with development of *Lepidosiren paradoxa* and *Protopterus annectens* (Kerr 1909) and for a revision of Semon's (1893) studies on *N. forsteri*. Fertilised eggs of *N. forsteri* are hemispherical, 3-3.5 mm in diameter, telolecithal and green or brown in colour, with dark pigment granules in the cortex. The first three cleavages are usually meridional, followed by a latitudinal cleavage and then by increasingly irregular and asynchronous divisions. The dorsal lip of the blastopore is initially an irregular curved slit, but develops into a smooth crescentic shape. The lateral lips draw close together before a ventral lip forms. The blastopore of the early neurula is a dorsoventrally orientated slit which develops into an oval anus connected with the nervous system by the neurenteric canal until stage 34. Primary brain divisions and optic vesicles are present before the neural folds close. The neural rudiment forms as a tube and always retains this structure.

Morphogenesis of the nares is closely linked to the development of the upper lip. The lateral line system of the trunk first appears at stage 41 and at stage 43-44 on the head. By stage 45 the main lines of the head are visible and by stage 47, all lines have developed into a series of pits. The pronephros shows externally from stage 27 until 35 and is a useful guide to development between these stages. Until stage 31, the endoderm cell mass retains its globular shape while the head grows forwards, but this shape is progressively lost after stage 32 when the body axis lengthens and the tail bud grows. At stage 37-38 endodermal cells still laden with yolk globules are packed together in platelet fashion. The amount of yolk is noticeably reduced by stage 43, although a little is still present at stage 47 when the spiral valve becomes visible. Yolk has entirely disappeared by stage 49.

Pectoral fins appear at stage 42 in lake fish (44 in river fish) and become pigmented at stage 43. Fin skeletons are visible at stage 47. Pelvic fins follow a similar course of development but appear later, at stage 48 in lake fish, and at stage 50 in river fish. Dorsal and ventral medial fins start to develop at stage 35 when the axis of the body begins to extend. The dorsal fin extends as far forwards as the back of the head at stage 38 and then regresses so that it reaches only as far as the middle of the trunk at stage 54. A large pre-anal ventral fin, which never develops a skeleton, is present from stages 40-53, reaches a peak at stage 49 and is then resorbed. Scales do not appear until stage 52, and are well established by stage 54. Melanin appears at stage 36 under the epidermis, and melanophores are widespread by stage 42. Cells containing a red pigment are present at stage 43.

There is no obvious metamorphosis. The adult form develops gradually over a period of months.

INTRODUCTION

The Queensland lungfish, *Neoceratodus forsteri* (Kreffft) was regarded as a giant amphibian when it was first discovered (Kreffft, 1870). Though it was soon clear that it was a fish, (Günther, 1871) its embryonic development closely resembles that of the Urodela.

Semon (1893) described the external develop-

ment of *N. forsteri*, based on observations of eggs and larvae. In the present study a comparison of eggs and embryos collected in successive seasons and observation on living specimens as they developed have added new information. For this reason a revised version of the external development of *N. forsteri* is presented which differs in some details from that of Semon (1893).

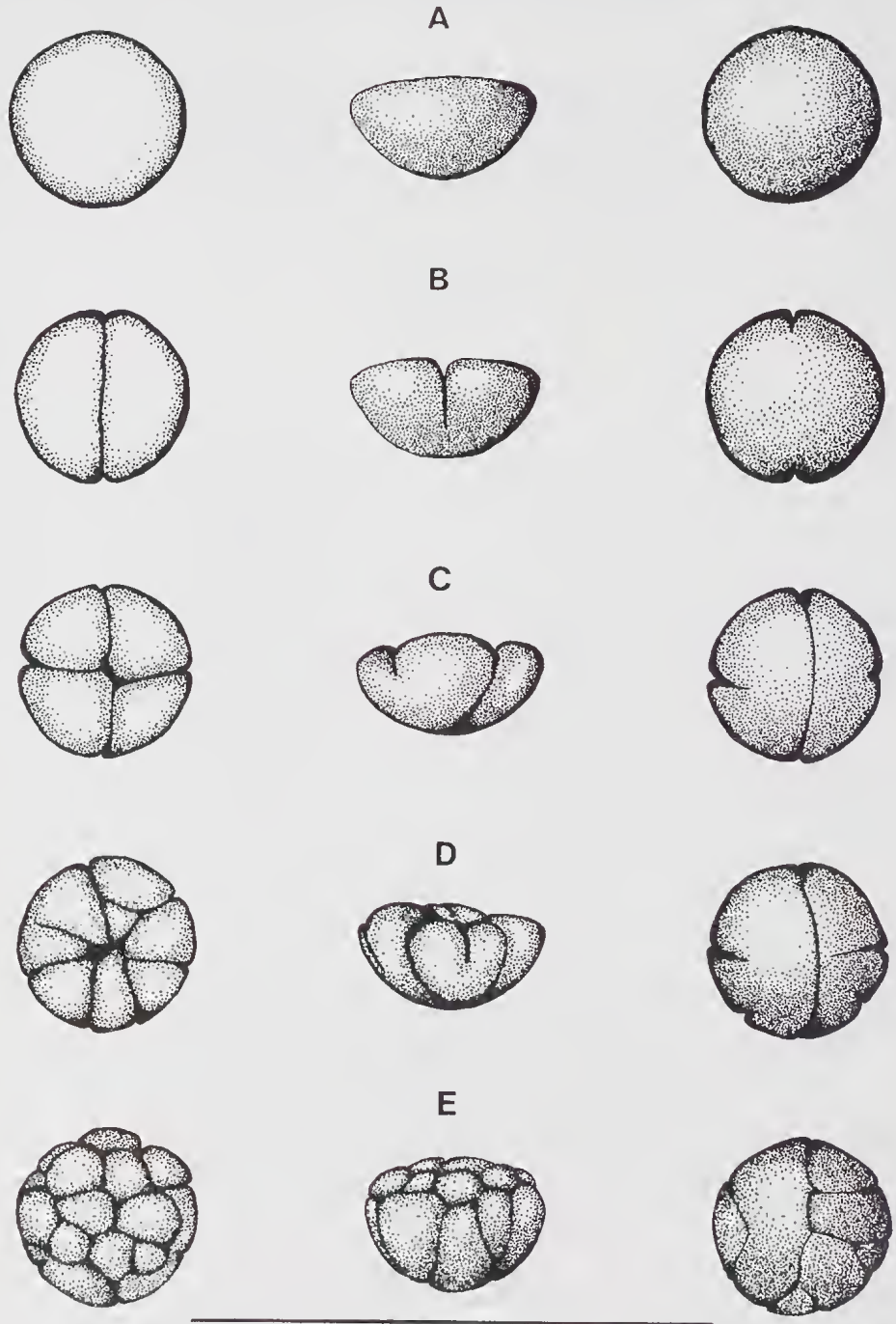


FIG. 1. Normal series of divisions in early cleavage in *Neoceratodus forsteri* eggs, stages 1-5, drawn with a camera lucida from fixed specimens removed from the vitelline and albumen membranes. The flat surface is shown on the left, side view in the centre and the convex surface on the right. A, stage 1, uncleaved egg; B, stage 2, first meridional cleavage; C, stage 3, second meridional cleavage at right angles to the first; D, stage 4, the third meridional cleavage, slightly irregular, with furrows on the convex surface lagging but usually dividing the cells from apex to base; E, stage 5 showing the first latitudinal cleavage, loss of the hemispherical shape as the segmentation cavity develops and delayed furrows in the convex surface. Scale line = 1 cm.

The stages of development given here for *N. forsteri* correspond closely to the stages described by Kerr (1909) for *Lepidosiren paradoxa* and *Protopterus annectens*.

MATERIALS AND METHODS

Eggs of *N. forsteri* were collected from Enoggera Reservoir, Brisbane, Queensland, (1969–1974) and from the Brisbane River over 3 seasons 1977–1979, and reared under the conditions described by Kemp (1981).

Most of the following description has been based on observations of living specimens as they developed, and of a series of fixed eggs, embryos and larvae. Living eggs were observed at frequent intervals during development at temperatures of 18°C, 20°C, and 22°C.

Embryos were removed from the membranes and fixed in 5% neutral buffered formalin. A series of fixed embryos from stages 20–39 were dehydrated in alcohol and cleared in cedar wood oil to show internal detail. The descriptions are based largely on eggs from Enoggera Reservoir and eggs from the River are described when they are different.

RESULTS

THE EGG

The egg is telolecithal, enclosed in a jelly capsule (outer albumen) about 2–3 mm thick. This is sticky when first laid, and allows the egg to adhere to water weeds. The external surface of the albumen soon becomes covered with debris and microscopic plant and animal life and the jelly loses its stickiness unless it is accidentally detached from the weed, when the freshly-exposed layer of albumen is adhesive again.

Within the outer albumen membrane there are two membranes, the inner albumen and the vitelline respectively. They are closely applied to each other but clear of the egg surface (P1. 1A). The fluid filled space enclosed by the membranes is spherical and the diameter slightly larger than that of the egg itself (P1. 1A).

The fertilised uncleaved egg from Enoggera Reservoir is hemispherical, 3–3.5 mm in diameter (Fig. 1A; P1. 1A), and lies free in the space formed by the vitelline membrane with the flatter side uppermost and the convex surface down. The egg is very delicate and the cytoplasm yolk laden. Eggs may be green or brown in colour depending on the colour of the yolk granules. Some dark pigment is present in the cortex of the egg, either on the flat surface, or on the convex surface or divided between the two (P1. 1C and 1J). A spot,

paler than the ground colour of the egg, lies within the pigmented area of a number of eggs (P1. 1A, D and J). In some eggs an irregular dark area (not associated with the pigment patch) was also present (P1. 1A).

River eggs are rounder than lake eggs but are otherwise similar.

CLEAVAGE

Cleavage in *N. forsteri* is so often irregular that it is difficult to describe a normal series. Figs. 1 and 3, and P1. 1C–I illustrate the 'normal' sequence and Fig. 2 and P1. 1J–L some common variations. Cleavage stages are summarised in Table 1.

Stages 2–6 inclusive are cleavage stages (P1. 1D–F and Figs. 1B–E and 3A). First cleavage is meridional, usually dividing the egg into two equal parts, and normally appears first at the pigmented uppermost surface and passes to the opposite side (Fig. 1B). First cleavage divides the pigment and the pale spot if present, but the cleavage products do not necessarily get an equal share of either even if they are equal in size themselves. Sometimes the pale spot is not divided (P1. 1J).

When first cleavage has been equal, the second cleavage furrow (stage 3) is also meridional and will normally appear at right angles to the first and pass from the flat pigmented surface to the rounded unpigmented surface (Fig. 1C). Each of the resulting cells has some pigment and usually a portion of the pale spot if originally present (P1. 1D). The small dark area of some eggs (P1. 1A) disappears.

The previously mentioned flat pigmented area may take on pronounced rounded contours in living eggs at this stage, though it remains uppermost in the intravitelline space. Presumably that part of the egg which contains the larger yolk granules is heavier and remains beneath, pressed against the vitelline membrane.

The third cleavage, also meridional, cuts each quarter of the egg down the middle usually radially but sometimes at right angles to this (stage 4, Fig. 1D and P1. 1E). Pigment retains its original position on the egg surface but is now divided between a number of cells, often unevenly. The pale spot is difficult to distinguish.

The fourth cleavage (stage 5) is the first latitudinal one and usually produces eight micromeres and eight macromeres (P1. 1F and Fig. 1E). The micromeres have most of the egg pigment and are uppermost in the intravitelline space. The egg is more rounded on top, owing to

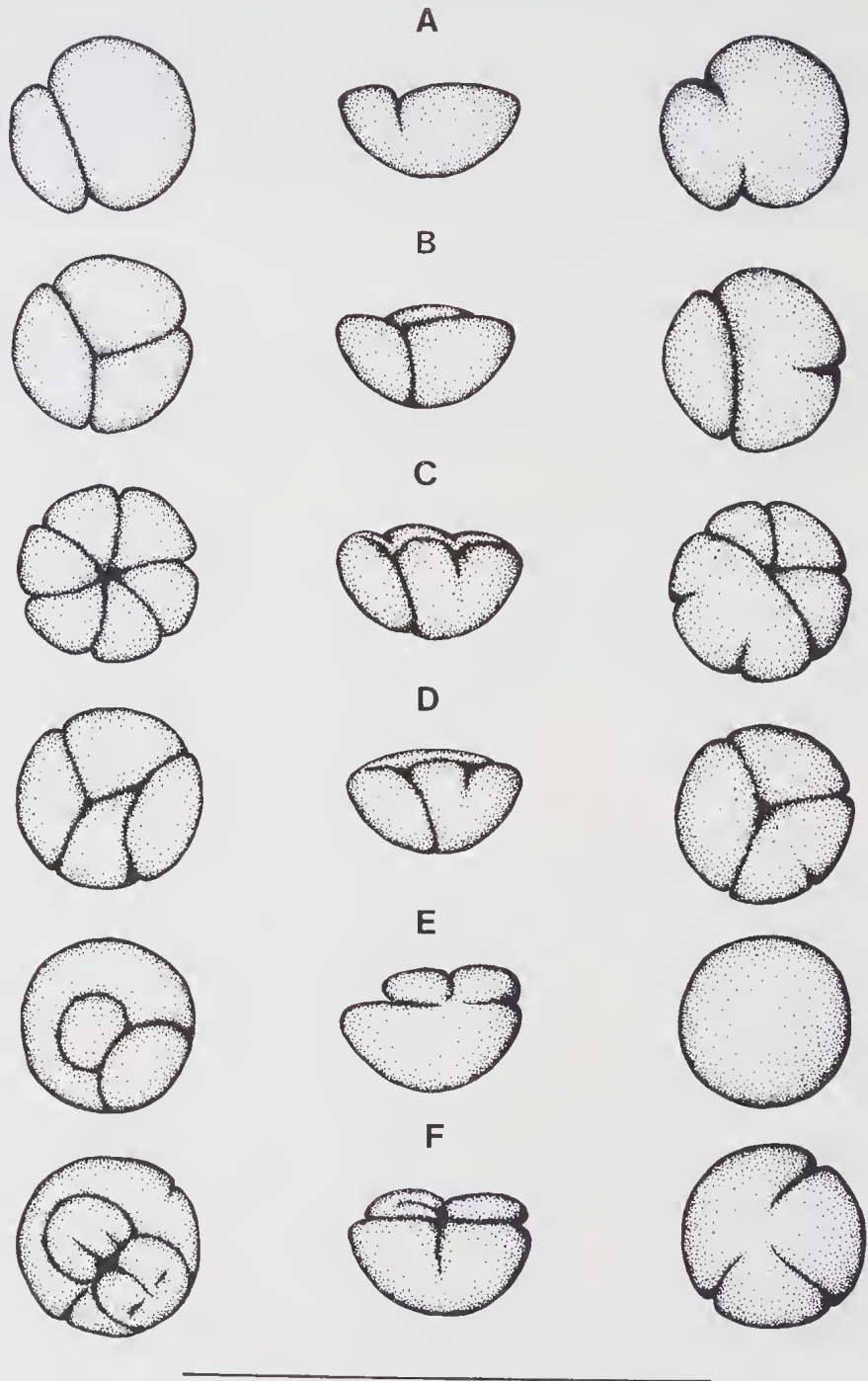


FIG. 2. Abnormal cleavages in a series of fixed eggs removed from the vitelline and albumen membranes before drawing. The flat surface is shown on the left, side view in the centre, and the convex surface on the right. A, common form of unequal first division; B, an egg divided into three equal cells; C, an egg with six equal cells; D, cleavage of Semon's stage 5 (1893, table 1, fig. 50); E, an abnormal division resulting in a button of small cells on top of uncleaved yolky material; F, later cleavage of an egg of the type illustrated in Fig. 2E; the small cells have divided several times and a few cleavage furrows have appeared in the convex surface. Scale line = 1 cm.

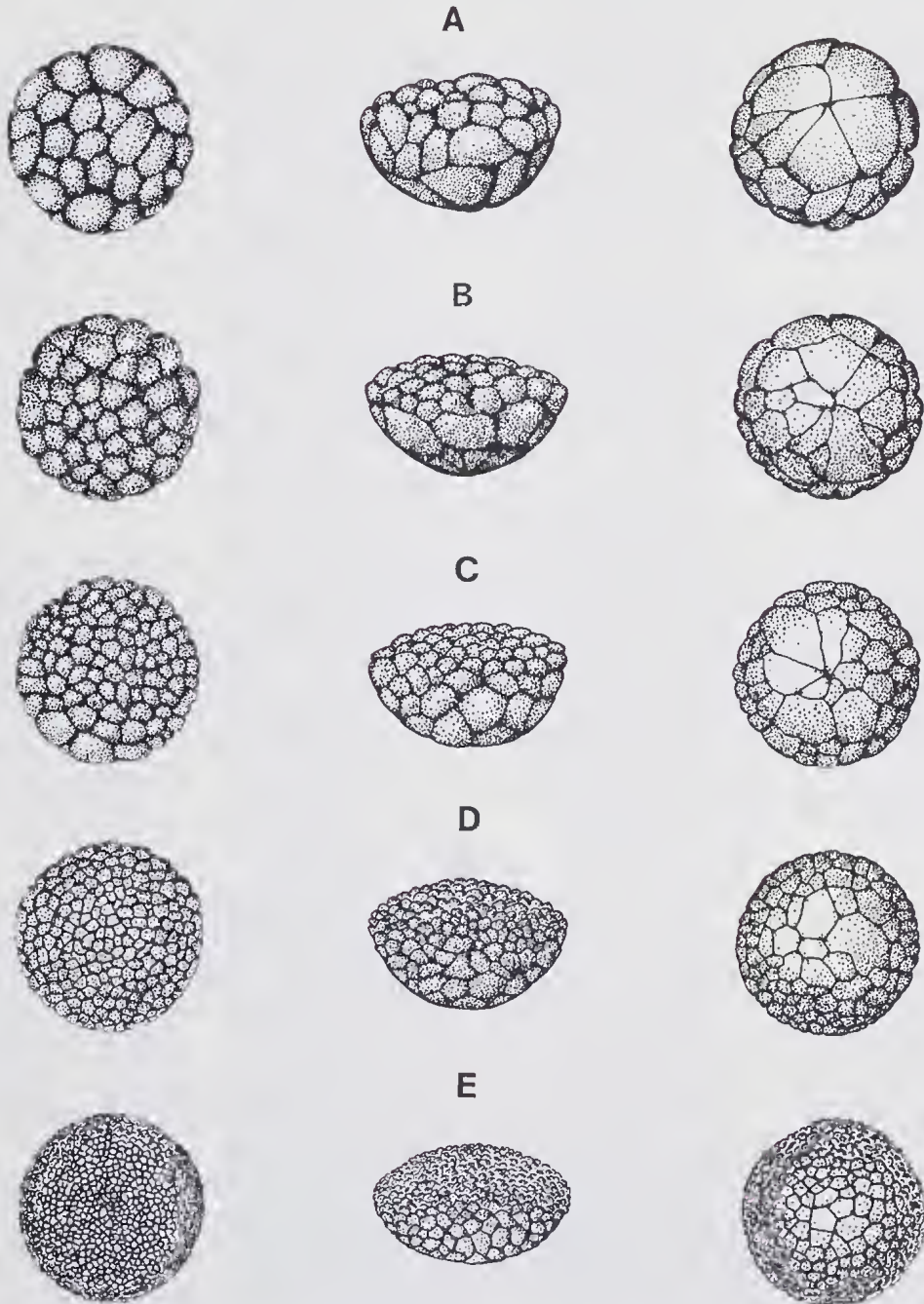


FIG. 3. Normal late cleavage stages and blastulae in *N. forsteri*, drawn from fixed specimens removed from the membranes. The flat surface is shown on the left, side view in the centre and convex surface on the right. A, stage 6, more horizontal and vertical cleavages; B, stage 7, cleavage continues more quickly among the smaller pigmented cells than in the heavily yolked cells of the convex surface; C, stage 8, large celled blastula; D, stage 9, a medium celled blastula; E, stage 10, a small celled blastula. The segmentation cavity (blastocoel) increases in size. Cells of the convex surface are flattened against the vitelline membrane (pavement cells), and the pigmented cells of the sides and roof of the blastocoel project into the intravitelline space and have rounded contours. Scale line = 1 cm.

the development of the segmentation cavity or blastocoel as space appears between the blastomeres. Cell division subsequently becomes asynchronous.

The fifth cleavage (stage 6) occurs quickly in the micromeres and is vertical. It is slower in the macromeres and here it tends to be latitudinal. The segmentation cavity increases in size (Fig. 3A).

Common variations of this pattern include gross disparity in the sizes of the cleavage products (Fig. 2A), and in the initiation of a cleavage furrow in the unpigmented rounded surface of a normally pigmented egg or in the pigmented rounded surface of an abnormally pigmented egg (Pl. 1J). Sometimes a latitudinal division occurs first (Fig. 2E).

If cleavage was unequal in the first division the smaller cell often divides first. A less frequent variation is the division of the larger cell to produce three cells of equal size (Fig. 2B), resulting in six equal sized cells instead of eight (Fig. 2C). Several eggs cleaved unevenly in a manner reminiscent of Semon's stage 5, with two large cells flanking two smaller cells divided across the centre of the egg (Fig. 2D and Pl. 1K).

When first cleavage was latitudinal (Fig. 2E) the small cell on top of the uncleaved yolk continued to divide rapidly. The yolk mass lagged considerably (Pl. 1L and Fig. 2E and F).

FORMATION OF THE BLASTULA

The blastula forms during stages 7-11 (Figs. 3B-E, 4A; Pl. 1G, H, I; see Table 2). The cells continue to divide and the previously flat surface becomes more rounded as they project into the intravitelline space. Cells in the convex region are pressed against the membrane and resemble a pavement. The segmentation cavity enlarges slowly until stage 11, when it expands rapidly to fill the entire intravitelline space. The cells of the thin walls and roof of the blastocoel are still rounded but those at the base are flattened against the vitelline membrane. The egg cannot rotate in the intravitelline space.

As the blastula develops it becomes clear that the position of the micromeres is correlated with the position of the egg pigment on the surface of the egg when it started to cleave, i.e. unusually placed pigment means unusually placed micromeres. This type of abnormality is smoothed over by stage 11 as cells move and a normal blastula is formed.

FORMATION OF THE GASTRULA

The gastrula forms during stages 12-16 (Figs. 4B-F, Pl. 2A, B). The earlier stages are prolonged (12, 13 and 14) but the process is completed quickly.

While gastrulation in *N. forsteri* usually follows the sequence of stages shown in Fig. 4, observations on living eggs reveal more variability e.g. an embryo in which formation of the anterior neural plate is advanced may also have a round yolk plug (7.5% of embryos). Such variation does not usually reduce the viability of the embryos — the yolk plug closes eventually and a normal neurula is formed. As with cleavage, 'abnormalities' are common and do not block development, unless they are extreme. The most common condition of gastrulation is described from a series of fixed embryos.

Between the small pigmented cells which cover the expanded blastocoel and the pavement cells of the base, an irregular slightly curved slit develops (Pl. 2A and Fig. 4B). Rounded cells have begun to invaginate and appear to be pulling in the pavement cells. The crescentic dorsal lip which develops from this slit (Fig. 4C) is more curved and smoother in outline. The pavement cells which have continued to divide take up less of the external area of the egg. At the outer limits of the crescent, rounded cells are being drawn inwards (Figs. 4C and D).

Next, the lateral lips form and draw together. Pavement cells are confined to the central area, under the dorsal lip, and more cells with pigment are involved in invagination (Fig. 4D). The lateral lips draw together but there is still no sign of a ventral lip except for a line of slightly indented cells where it would be expected to form (Fig. 4E). The external surface of the egg is entirely covered by pigmented cells and the pavement cells have disappeared. The blastocoel is smaller.

The ventral lip finally appears as a short section between the two lateral lips which have almost met in the midline (Pl. 2B and Fig. 4F). The blastopore is now slit-like dorsally where the lateral lips have met. The blastocoel is reduced in size, the egg is no longer spherical and there is sufficient space between the egg surface and the vitelline membrane for the embryo to rotate freely again. From the dorsal part of the blastopore runs a diffuse double streak of deeply pigmented cells which mark the position of the future neural plate. This part of the egg is also slightly flattened (Fig. 4F).

TABLE 1: CHARACTERISTICS OF STAGES 1-6.

Stage	External Features	Distribution of Pigment	Segmentation Cavity	Stage (Semon 1893)	Stages (Kerr 1909)
1 (Fig. 1A; Plate 1A & C)	hemispherical, green or light brown in colour	irregular patch, on flat surface	—	—	—
2 (Fig. 1B)	hemispherical, first meridional furrow forms	irregular patch on flat surface of each cell	—	2	2
3 (Fig. 1C, Plate 1D)	hemispherical, second meridional furrow forms at right angles to first	shared roughly among the cells, on previously flat surface	—	4	3
4 (Fig. 1D, Plate 1E)	flat surface grows rounder; third meridional furrow bisects each cell	shared roughly among the cells, on previously flat surface	—	6	4
5 (Fig. 1E, Plate 1F)	egg more rounded, first latitudinal furrow appears	smaller cells have pigment, larger ones have none	small space in centre	7	—
6 (Fig. 3A)	flat surface furrows are vertical, convex horizontal; division no longer synchronous	pigment confined to micromeres	larger central space	9	7
					VII

L. paradoxa
P. annectens

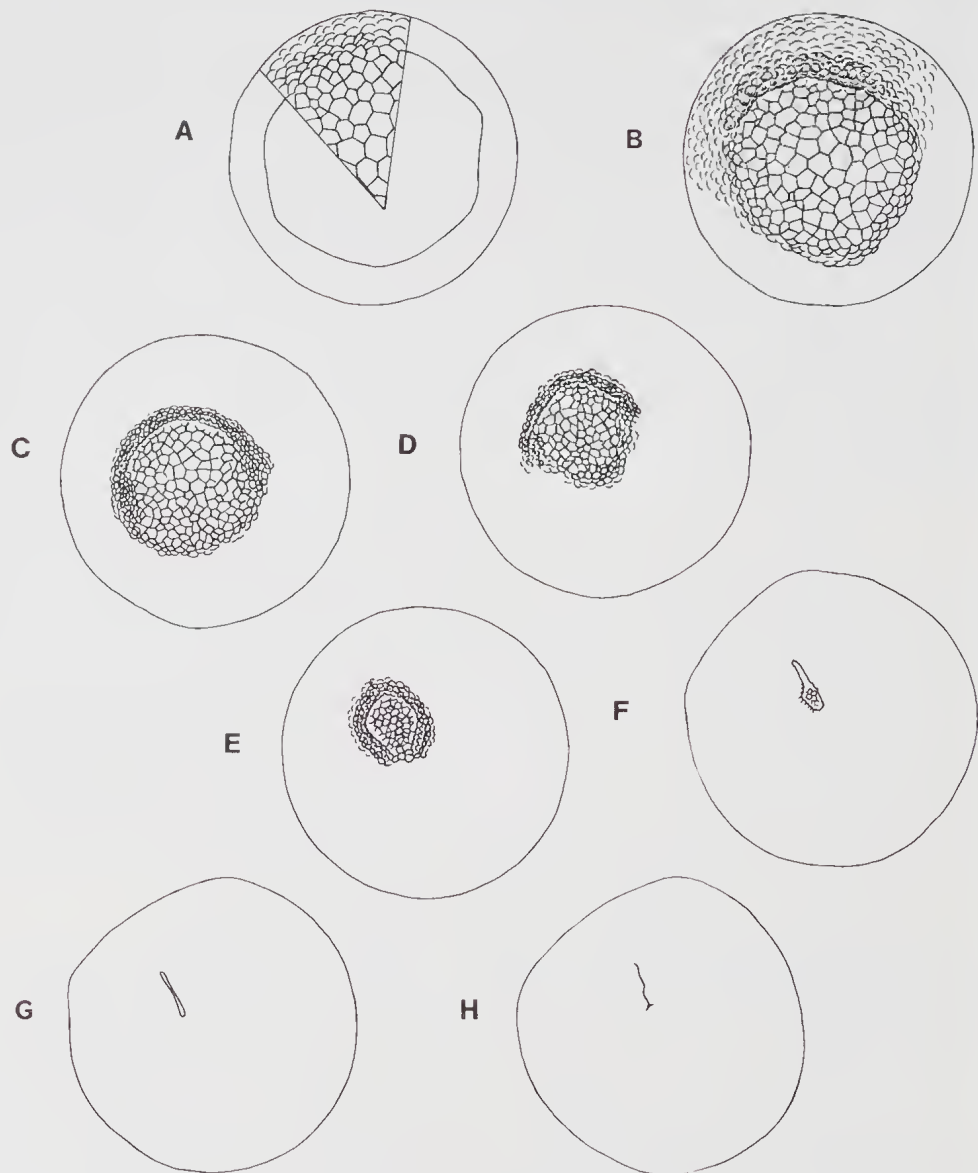


FIG. 4. Formation of the gastrula in *N. forsteri*. A series of fixed eggs drawn without removal from the membranes, because the blastocoel is now so large that it fills the entire intravitelline space and the egg cannot be removed without damage. All are views of the blastopore region and show details of the behaviour of the pavement cells and of the pigmented cells enclosing the blastocoel cavity. A, stage 11, showing the gradation from the pavement cells at the base of the egg to the cells at the sides of the blastocoel cavity which are smaller and rounder in shape. The solid line represents the limit of the pavement cells; B, stage 12, with an irregular slit shaped blastopore at the junction of the pavement and the pigmented cells. Cell division has continued and invagination involves pigmented cells rolling over the pavement cells; C, with a crescent shaped blastopore and invagination at the lateral lips as well as at the dorsal lip. The pavement cells are smaller and fewer in number; D, stage 14, with well formed lateral lips drawing closer together giving the blastopore the appearance of a wide V. Most of the pavement cells have been drawn inside; E, stage 15, still without a ventral lip, and with lateral lips curving round to meet each other; F, stage 16, in which the lateral lips have closed dorsally but are still separated ventrally, where a ventral lip has finally appeared; G and H, showing the variation in shape of the blastopores of neurulae, sometimes a closed dorsoventral slit, sometimes open. Scale line = 0.25 cm.

TABLE 2: CHARACTERISTICS OF STAGES 7-11

Stage	External Features	Distribution of Pigment	Segmentation Cavity or Blastocoel	Stage (Semon 1893)	Stages (Kerr 1909) <i>L. paradoxa</i> <i>P. annectens</i>
7 (Fig. 3B, Plate 1G)	cleavage vertical in small pigmented cells, horizontal in large ones	confined to micromeres	space between cells in centre of egg	—	—
8 (Fig. 3C, Plate 1H)	irregular and asynchronous cleavage continues; egg rounded	confined to micromeres	space between cells in centre of egg	10	—
9 (Fig. 3D, Plate 1I)	irregular and asynchronous cleavage continues; egg rounded	confined to micromeres	segmentation cavity begins to expand	—	—
10 (Fig. 3E)	cleavage continues, large yolk unpigmented cells pressed against vitelline membrane	confined to micromeres	expands further	11	IX
11 (Fig. 4A)	egg spherical, small pigmented cells form roof of blastocoel, large unpigmented yolk cells pressed against vitelline membrane	confined to small cells forming roof and sides of blastocoel	attains greatest size	—	9

THE FATE OF THE BLASTOPORE

In a young neurula of stage 17, the blastopore is a dorsoventral slit at the posterior extremity of the neural folds (Figs. 4G and H, Pl. 2C). The cleared specimen of stage 21 has a partially divided blastopore (Fig. 6A) but the opening is single in the next stage illustrated (Fig. 6B). The individual of stage 23 (Fig. 6C) has two openings which are quite distinct, one within the neural folds and one just posterior to it. By stage 24, the blastopore, which is single in this specimen, lies inside the neural folds (Fig. 6D). As development proceeds the blastopore grows forward (still within the neural folds), and takes on an oval shape (stages 25–27, Figs. 6E, 7A and B). By stage 27 it has joined the gut cavity. When the neural folds finally close posteriorly a neurenteric canal lined with pigment granules is formed which lengthens as the anus grows forward (stages 27–30, Pl. 2G and H, an Figs. 7B–E). The neurenteric canal persists until stage 34 (Fig. 8D). The behaviour of the blastopore is summarised in Tables 3, 4 and 5.

FORMATION OF THE NEURAL FOLDS AND EARLY DEVELOPMENT OF THE BRAIN

This phase of development includes stages 17–30 (see Pl. 2D, E and Figs. 5–7). The neural plate has begun to form by stage 16 before the blastopore is fully developed. The presumptive neural plate flattens at stage 17 (Fig. 5B), and two streaks of darkly pigmented cells extend forwards from the blastopore. By stage 18 the folds are raised anteriorly (Fig. 5C and Pl. 2D). Elevation of the folds and formation of a deep median furrow between the two lines of pigment continues, and at stage 19, the neural folds are easily recognised (Fig. 5D). They are wide anteriorly, with a distinct transverse crest, and low and narrow posteriorly.

This is the stage at which Semon's ectodermal median suture shows occasionally having been found only in the embryos of one season out of nine. Possibly the furrow is a less definite ectodermal median suture without the deep internal folding characteristic of the latter (Semon 1901b; Figs. 7 and 8).

In eggs of stage 20 the transverse crest is deeper and three bilateral swellings, corresponding to the three primary vesicles of the brain, are already present, although the neural folds are still wide open (Fig. 5E). These show best in living eggs, and are well formed by stage 21 (Fig. 6A). The transverse crest sinks inwards and disappears as the two parts of the brain draw together (stage 23,

Pl. 2E and Fig. 6C). The median furrow disappears.

Epithelium covers the anterior neural folds soon after they meet (stage 24, Fig. 6D) and the process continues backwards. The posterior part of the folds is still open at stage 25 (Fig. 6E) and the tip around the blastopore/anus does not close until stage 26 (Fig. 7A and Pl. 2F).

Cleared specimens show that the nervous system of *N. forsteri* forms initially as a tube and never loses its tubular nature. The hollow cavity is lined with granules of egg pigment contained in the part of the nerve cells closest to the cavity. This dark lining persists for a long time (cf. Greil 1908). Formation of the nervous system is summarised in Tables 4, 5 and 6.

DEVELOPMENT OF THE EYE, AUDITORY APPARATUS AND OLFACTORY ORGAN

The optic vesicles can first be distinguished as a paired hollow outgrowth of the fore brain in cleared specimens of stage 23 (Fig. 6C). By stage 30, the eye is visible externally and a lens is present by stage 32. Pigment appears in the eye at stage 35 just before the rest of the body becomes pigmented.

The auditory apparatus first appears at stage 25 as a deeply pigmented shallow pit on each side of the head (Pl. 2F and Fig. 6E). The pits grow deeper (stages 26 and 27, Figs. 7A and B), sink inwards and become covered by epithelium (stages 28 and 29, Figs. 7C and D). The nervous connection with the brain is present at stage 30 (Fig. 7E) and the ductus endolymphaticus is recognised in cleared specimens by stage 34 (Fig. 8D).

Primordia of the olfactory organ are late to appear. The placodes form as slight depressions on the anteroventral surface of the head at stage 30 (Fig. 7E). They sink inwards and by stage 31 each has a single opening on the external surface rostral to the developing mouth (Fig. 8A). As the upper lip develops, the nares are found at either end of a deep groove at the centre of which is the mouth (stages 37 and 38 — Figs. 10A and B). As the mouth develops the nares are drawn further inwards. The development of the olfactory organs of *N. forsteri* has been described by Bertmar (1965).

Formation of the sense organs is summarised in Tables 5 and 6.

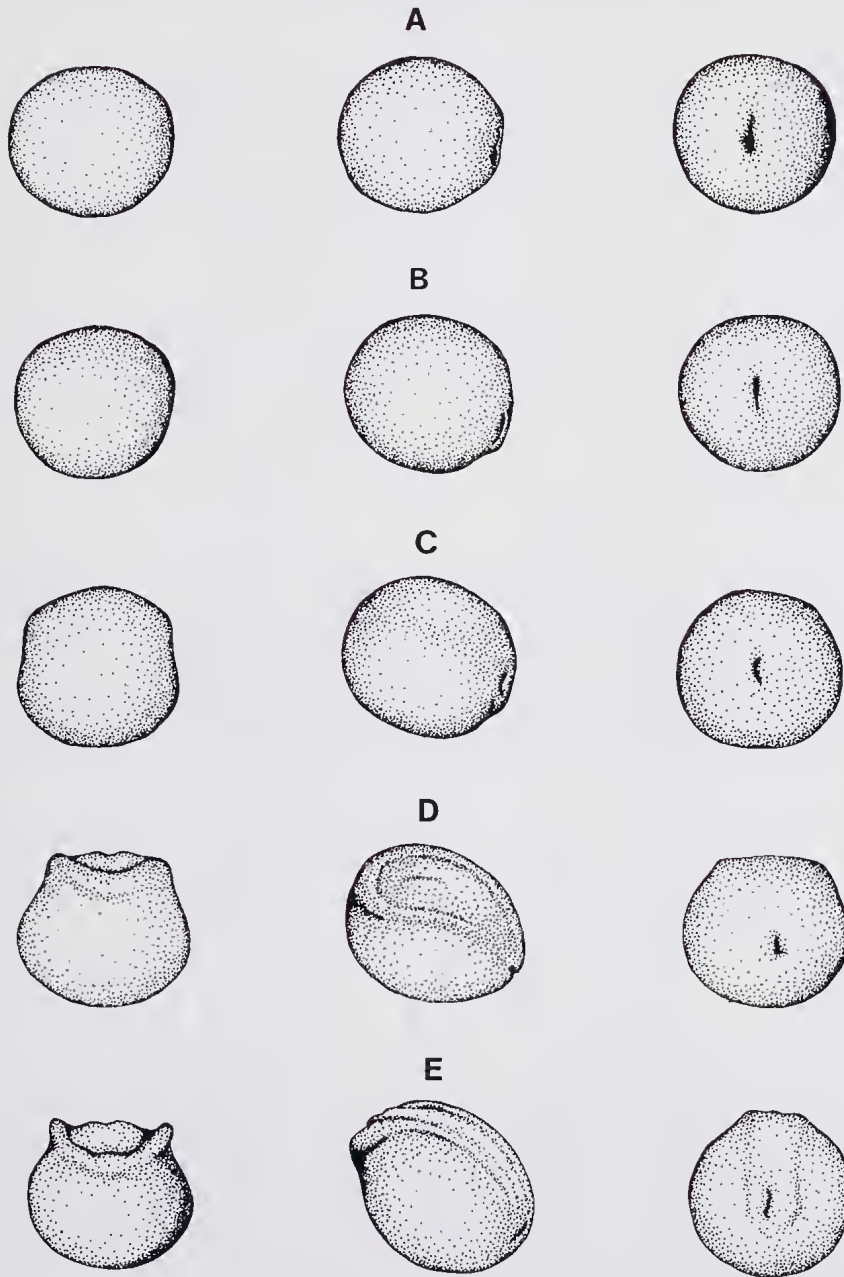


FIG. 5. Early development of the neurula in *N. forsteri*. A series of fixed eggs drawn after removal from the membranes. An anterior view is shown on the left, lateral in the centre and posterior on the right. A, stage 16, showing the almost closed blastopore and the flattened area of the presumptive neural plate; B, stage 17, which is more elongated with a flatter presumptive neural plate and a slit-like blastopore; C, stage 18, showing the lifting of the neural folds anteriorly and the apparently double blastopore; D, stage 19, with neural folds forming anteriorly and a shallow cleft in the centre of the neural plate; E, stage 20, in which the transverse crest and primordia of the primary brain vesicles are visible in anterior view. The blastopore appears to be surrounded by presumptive neural material. Scale line = 1 cm.

TABLE 3: CHARACTERISTICS OF STAGES 12-16

Stage	External Features	Distribution of Pigment	Blastocoel	Blastopore	Stage (Semon 1893)	Stages (Kerr 1909) <i>L. L. paradoxa annectens</i>
12 (Fig. 4B, Plate 2A)	spherical	present on cells forming roof and walls of blastocoel	very large	long ragged slit at junction of unpigmented yolky cells and small pigmented cells	12	11 XI
13 (Fig. 4C)	spherical	present on cells forming roof and walls of blastocoel	very large	crescentic, position as above; some yolky cells invaginated	13	—
14 (Fig. 4D)	spherical	present on cells forming roof and walls of blastocoel	a little smaller	Broad V-shape, most yolky cells invaginated, as well as some cells with pigment, lateral lips slightly drawn in	14	13 XIII
15 (Fig. 4E, Plate 2B)	spherical	all external cells are pigmented, also some invaginated cells; pigment beginning to concentrate in region of future neural plate	a little smaller	lateral lips closing, yolky cells inside, no ventral lip	—	14 XIV
16 (Fig. 4F)	slightly flattened in region of future neural plate	double streak of pigment in region of future neural plate	reduced	lateral lips meet in mid-line dorsally, separate ventrally; ventral lip present	16	—

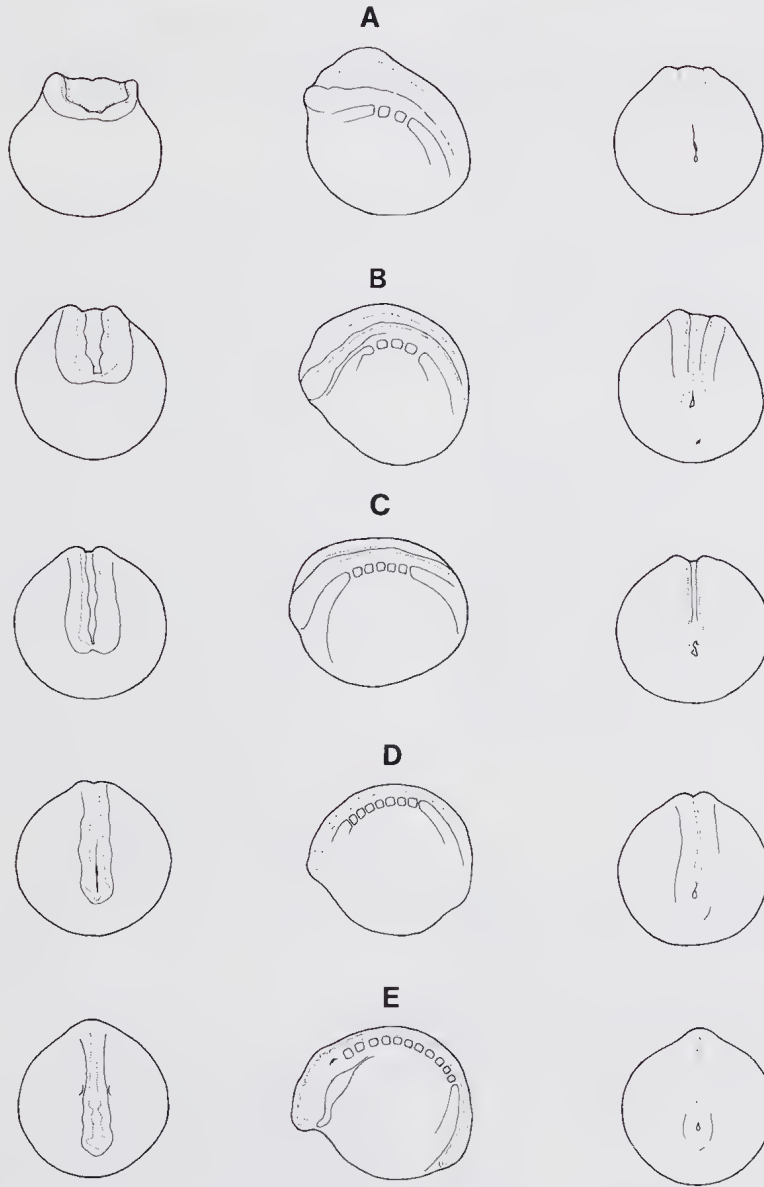


FIG. 6. Drawings of cleared eggs showing later development of the neurula. The anterior view is shown on the left, lateral in the centre and posterior on the right. A, stage 21, with the neural folds closer anteriorly than posteriorly, blastopore partly outside the neural folds, transverse crest and fore, mid and hindbrain vesicles, and two somites; B, stage 22, showing the distinct primary brain vesicles, transverse crest sinking inwards, 3 somites and the slit-like blastopore; C, stage 23, an embryo of 5 somites in which the neural folds are nearly closed anteriorly, the apparently double blastopore partly enclosed in the folds posteriorly and early indications of the opticoele; D, stage 24, with neural folds closed anteriorly and open posteriorly where they surround the now single blastopore; seven somites are present and the telencephalon, diencephalon, with associated opticoeles, mesencephalon, metencephalon, and myelencephalon can be distinguished; E, stage 25, in which ectoderm has covered all but the most posterior part of the neural folds, the opticoele is more distinct, neuromeres are present in the hindbrain, the otic placode has appeared, there are 11 somites and the oval blastopore is surrounded by neural material. The archenteron has expanded anteriorly to form the pharynx. Scale line = 1 cm.

DEVELOPMENT OF THE LATERAL LINE SYSTEM

The lateral line system of the trunk begins to form at stage 41 (Pl. 3B and Fig. 12A), and extends along the body (Figs. 12B and C and Table 8). At stage 44 it reaches the tail tip (Fig. 13A). There is a slight flexure in the tail region.

The development of the lateral lines and pits of the head of *N. forsteri* has been followed in reconstructions of developing embryos and larvae by Pehrson (1949). The present work is concerned only with those parts that are easily visible externally and therefore useful for estimation of the stage of development of larvae (Tables 8 and 9).

At stage 44 (fish from Enoggera Reservoir) and stage 43 (river fish) sensory lines appear above and below the eye (Fig. 13A). By stage 45 (Fig. 13B), the main lines of the head are established, as follows: in side view the supra- and infraorbital, in ventral view the mandibular and dorsally the temporal and posterior head lines (Pehrson 1949). The jugal line forms at stage 46 (Fig. 13C). By the next stage the supratemporal cross commissure has formed and the distinct lines have broken up into separate pits (Pl. 3D and Fig. 14A). The oral line is not visible until stage 49 (Fig. 15A). The lateral line system of the head is soon obscured by increasing pigmentation in the skin.

APPEARANCE OF THE MYOTOMES

In embryos of stage 20 the mesoderm is undivided. By stage 21, before the neural folds close, two somites are present (Fig. 6A) and somites are added in front and behind the first two, up to stage 24 (Fig. 6D), after which they are added only posteriorly. The V-shape of the myotomes develops by stage 33 (Fig. 8C). Numbers of somites at the various stages are included in Tables 4, 5, 6 and 7.

THE PRONEPHROS

The pronephros is distinguishable externally as a prominent bulge just behind the auditory placode from stage 27 (Fig. 7A and Table 5) onwards and in cleared specimens it is associated with postotic somites 4, 5 and 6 (stage 30, Fig. 7E and Table 6 and stages 31–34, Figs. 8A–D). At stage 32 it appears as two tubules and a short duct (Fig. 8B and Table 6), which has grown towards the tail by stage 33 (Fig. 8C). The whole organ has moved posteriorly in relation to the somites at stage 35 (Fig. 9A and Table 7). Subsequently it becomes difficult to distinguish externally and loses its usefulness as a guide to stage of

development. Further details of pronephric development may be found in the series of papers by Fox (1960, 1961 and 1962).

DEVELOPMENT OF THE MOUTH

Up to stage 28, there is little sign of mouth development. The presumptive mouth region is smooth and uncontoured until stage 29, when an indentation develops on the anteroventral surface of the head (Fig. 7D). At stage 33 (Fig. 8C) a triangle of pale endoderm shows through the ectoderm of the mouth region. At stage 34 the mouth cavity grows deeper and at stage 35 the upper lip begins to form, gradually involving the nares at the lateral borders (Figs. 8D, 9A, 9B and 10A). The lower lip starts to develop at stage 38 as the mouth cavity grows deeper (Fig. 10B). At stage 41 the foregut and mouth cavities join. The lower lip grows forwards, meeting the upper at stage 45 (Figs. 12 and 13). The mouth shifts anteriorly, becoming terminal at stages 51–52 (Figs. 16A and B). By stage 54, differential growth has left the mouth in a slightly subterminal position, as in the adult, with the lower jaw fitting within the upper and the anterior openings of the nares showing inside the upper lip (Fig. 17B). Gular pits develop behind the lower lip at stages 46–47 (Fig. 13C and 14A).

These phases of development are summarised in Tables 6, 7, 8, 9 and 10.

FORMATION OF THE ARCHENTERON AND THE GUT

Towards the end of the period of neural tube formation (stage 25, Fig. 6E) the archenteron is wide anteriorly and very narrow behind. By stage 27 the posterior section is slightly distended. The anus is linked with the posterior end of the nerve cord as well as the gut (Fig. 7B). Anteriorly the foregut is growing forward but the mouth has not yet appeared. The liver diverticulum develops at stage 33 (Fig. 8C) and the neurenteric canal disappears at stage 34 (Fig. 8D).

Until stage 31, the endodermal cell mass remains globular and the head extends forwards, but after stage 32, the endodermal cell mass progressively loses its round shape as the body axis lengthens and the tail bud grows. At stages 37–38 (Pl. 3A) the endodermal cells, still laden with yolk, are packed together in platelet fashion and these show through the epidermis.

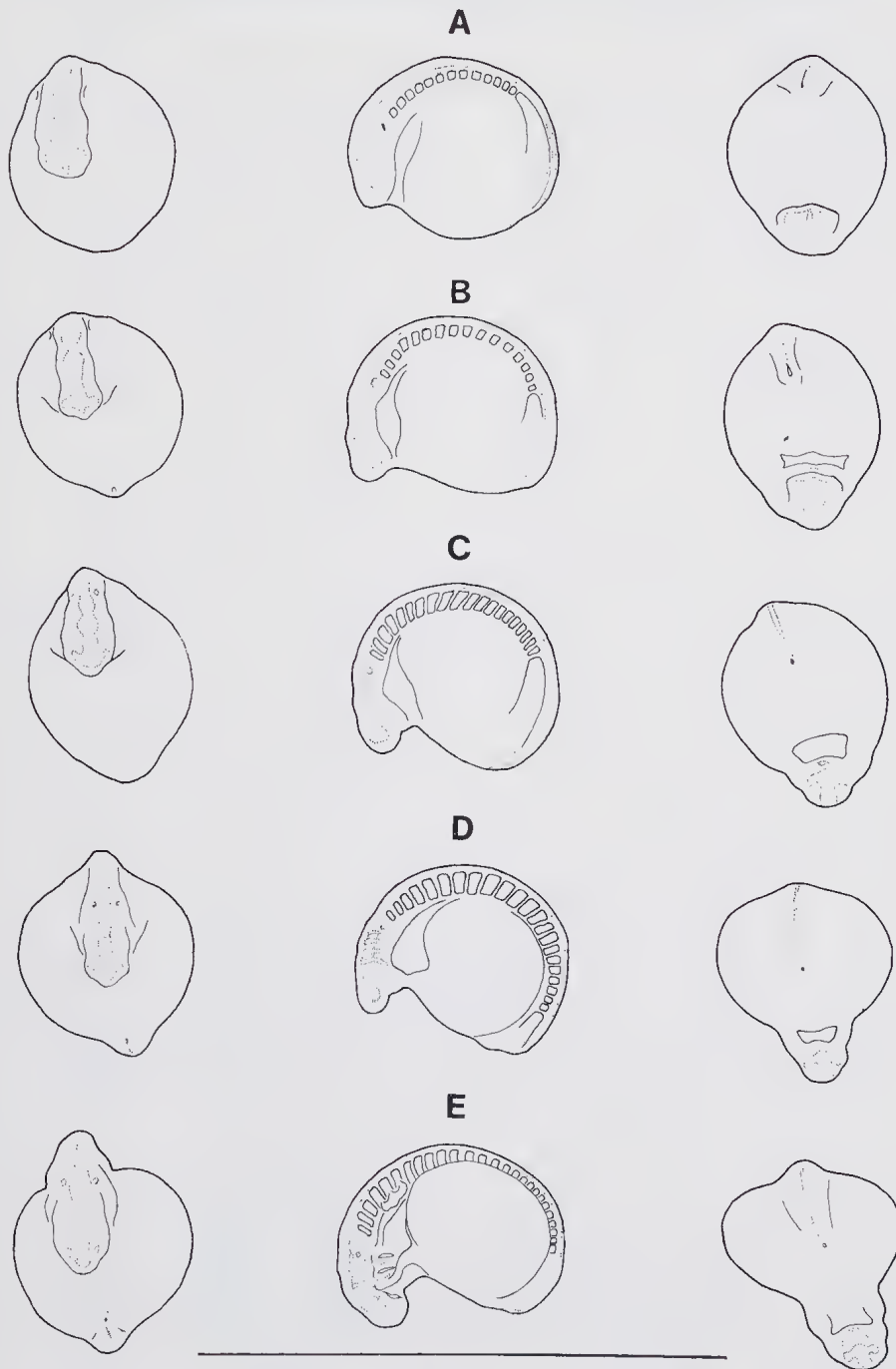


FIG. 7. Cleared embryos of *N. forsteri* showing development of the head region. Anterodorsal view on the left, lateral in the centre and ventral on the right. A, stage 26, with opticocoeles, otic placode, pronephros, 13 somites, neurenteric canal and pharynx. B, stage 27, with 16 somites and the blastopore (anus) continuous with the gut and the neural tube; C, stage 28 showing further development of the opticocoeles, formation of the otic capsule and 20 somites; D, stage 29 with dorsal root ganglia and 22 somites; the anus has grown forward along the mid-ventral line and the neurenteric canal has grown with it; E, stage 30, with 26 somites, 2 gill slits, olfactory placode close to the developing mouth and pronephros associated with post-otic somites 4, 5 and 6. Scale line = 1 cm.

TABLE 4: CHARACTERISTICS OF STAGES 17-22

Stage	Blastopore	Nervous System	Somites	Stage (Semon 1893)	Stage (Kerr 1909) <i>L. paradoxa</i> <i>P. annectens</i>
17 (Fig. 5B, Plate 2C)	single dorso-ventral slit; partly within neural folds	indicated by concentration of pigment cells and flattening of egg dorsally	—	—	—
18 (Fig. 5C, Plate 2D)	single dorso-ventral slit; partly within neural folds	greater concentration of pigment cells, more flattening, raising of neural folds anteriorly	—	17	XVI
19 (Fig. 5D)	single dorso-ventral slit; partly within neural folds	raising of neural folds progresses posteriorly, medium furrow in centre of plate in some eggs	—	18	—
20 (Fig. 5E)	single dorso-ventral slit; partly within neural folds	transverse crest and indications of the 3 primary brain vesicles are present; folds are wide open	block of undivided mesoderm	19	16
21 (Fig. 6A)	apparently double; upper opening in neural folds, lower outside	transverse crest, fore-, mid- and hind-brain readily visible	2	20	—
22 (Fig. 6B, Plate 2E)	single, not completely surrounded by folds	folds raised almost to blastopore and closer together, no medial cleft; transverse crest sinking, hind brain begins to divide into two parts	3-4 somites are formed at anterior and posterior ends of mesoderm block	22	—

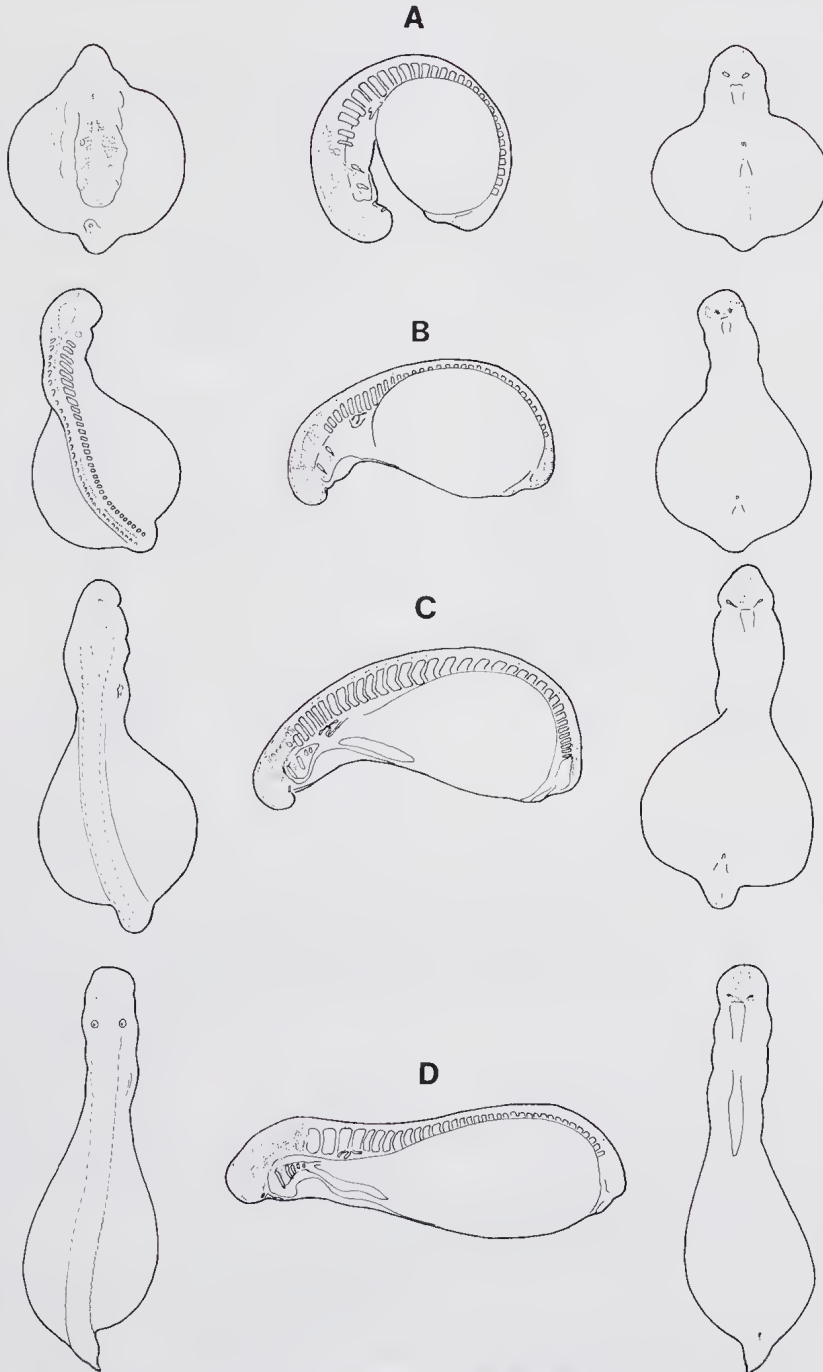


FIG. 8. Drawings of cleared specimens of late neurulae. Anterodorsal view on the left, lateral in the centre and ventral on the right. A, stage 31, in which all the structures present in stage 30 are larger and more defined, the endoderm is globular and there are 30 somites; B, stage 32, the embryo is growing longer with 33 somites and a pronephros with two tubules and a duct; C, stage 33, showing the developing mouth, the liver diverticulum of the foregut, four gill slits and 35 V-shaped somites; D, stage 34, with a distinct pronephros, 36 somites, neurenteric canal in the process of disappearing, a fifth gill slit, endolymphatic duct, deepening mouth cavity and developing nares. Scale line = 1 cm.

Yolk in endodermal cells is reduced considerably by stage 43 (Pl. 3C), although some yolk is still present at stage 47, when the form of the spiral valve becomes clear (Fig. 14A). Yolk has disappeared from the gut by stage 49, when the spiral valve is well formed (Fig. 15A). The rectum, which forms at right angles to the rest of the gut, shows clearly from stage 38 onwards (Fig. 10B). Development of the gut and associated structures is summarised in Tables 6, 7, 8 and 9.

THE GILL CLEFTS AND THE OPERCULUM

The gill clefts show externally at stage 30 (Fig. 7E) as they are situated on a prominent bulge on either side of the head and connect with the pharynx. There are 4 clefts by stage 33 and 5 at stage 34 (Figs. 8C and D). The operculum is present at stage 36 as a fold of epithelium between the first two gill clefts (Fig. 9B). The second cleft is covered at stage 37 (Fig. 10A) and the third at stage 38 (Fig. 10B). By stage 41 all four posterior slits are beneath the operculum (Fig. 12A). The operculum develops behind the first cleft and never covers it. Externally, the gill clefts do not yet appear to be open. At stage 44 the opercular folds meet midventrally (Fig. 13A).

FORMATION OF THE PAIRED FINS

Incipient pectoral fins are first recognised at stage 42 in lake fish (Pl. 3B and Fig. 12B) as tiny unpigmented primordia, one on each side of the body immediately behind the free edge of the operculum (Table 8). Pigment cells appear in the growing fin at stage 43 (Pl. 3C). By stage 45 the fins are larger and the anterior part is covered by backward growth of the opercular folds (Fig. 13B and Table 9). The skeleton of the fin can be seen in specimens of stage 47 (Fig. 14A). The pectoral fins do not appear until stage 44 in river fish.

The pelvic fins develop in a similar manner but appear slightly later, at stage 48 (Fig. 14B and Table 9) in lake fish. Pigment cells appear in the fin at stage 49 and the skeleton shows at stage 51 (Fig. 15C and Table 10). Pelvic fins do not appear until stage 50 in river fish.

DEVELOPMENT OF THE MEDIAL FINS

At stage 35 the tail bud (Pl. 2J and Fig. 9A) straightens and begins to grow larger. A dorsal median fin, one third of the length of the trunk, is present at stage 36 (Fig. 9B). By stage 37 there is a small median ventral fin and the dorsal fin, now half the length of the trunk (Pl. 3A and Fig. 10A), reaches its greatest length relative to the trunk at stage 38 (Fig. 10B), extending as far as the back

of the head; a pre-anal ventral fin also appears. All three fins grow in subsequent stages (Fig. 11–14). At stage 49, skeletal elements can be distinguished in both the dorsal and post-anal ventral fins, but they never develop in the pre-anal ventral fin, which grows forward as far as the operculum (Fig. 15A) by stage 49 and thereafter regresses. It disappears by stage 53 (Fig. 16B). The dorsal fin also undergoes some regression, extending forward only as far as the middle of the trunk at stage 54 (Pl. 3H and Fig. 17B). Development of the medial fins is summarised in Tables 7, 8, 9 and 10.

FORMATION OF THE SCALES

Scales appear late, at stage 52 (Table 10) beginning as primordia regularly distributed over the body surface under the epidermis. They are well established by stage 54. The scales illustrated by Semon in his larva of stage 45 have not been observed at an equivalent stage in the present material.

DEVELOPMENT OF PIGMENT

Adults of *Neoceratodus forsteri* are characterised by two main pigments, melanin and a red pigment. Melanin (within melanophores) is the first to appear (stages 36–37, Pl. 3A), becoming widespread over the body by stage 42. The melanophores have the property of expansion and contraction in response to external light and dark illumination while the animal is young (cf. *L. paradoxa*, Kerr 1900).

River juveniles are pale brown and mottled, (Pl. 3H), and lake juveniles are usually dark brown. Spots of intense dark pigment on the dorsal fin are common. Cells containing a different pigment, red in living animals and colourless in fixed specimens, appear at stage 43 (Pl. 3C). They are larger than the melanophores. At first they are confined to the dorsal surface but as more develop they spread ventrally until they are scattered all over the body. Pigment formation is summarised in Tables 7, 8, 9 and 10.

THE INTRAVITELLINE SPACE AND THE PROCESS OF HATCHING

There is little change in the dimensions of the embryo, apart from those alterations in shape associated with cleavage and development of the blastula, gastrula and the neurula, until the head begins to extend forwards, at about stage 28. The head continues to grow, but the vitelline membrane does not start to expand until stage 32. Though capable of movement when removed from the membranes, the embryo lies inert in the

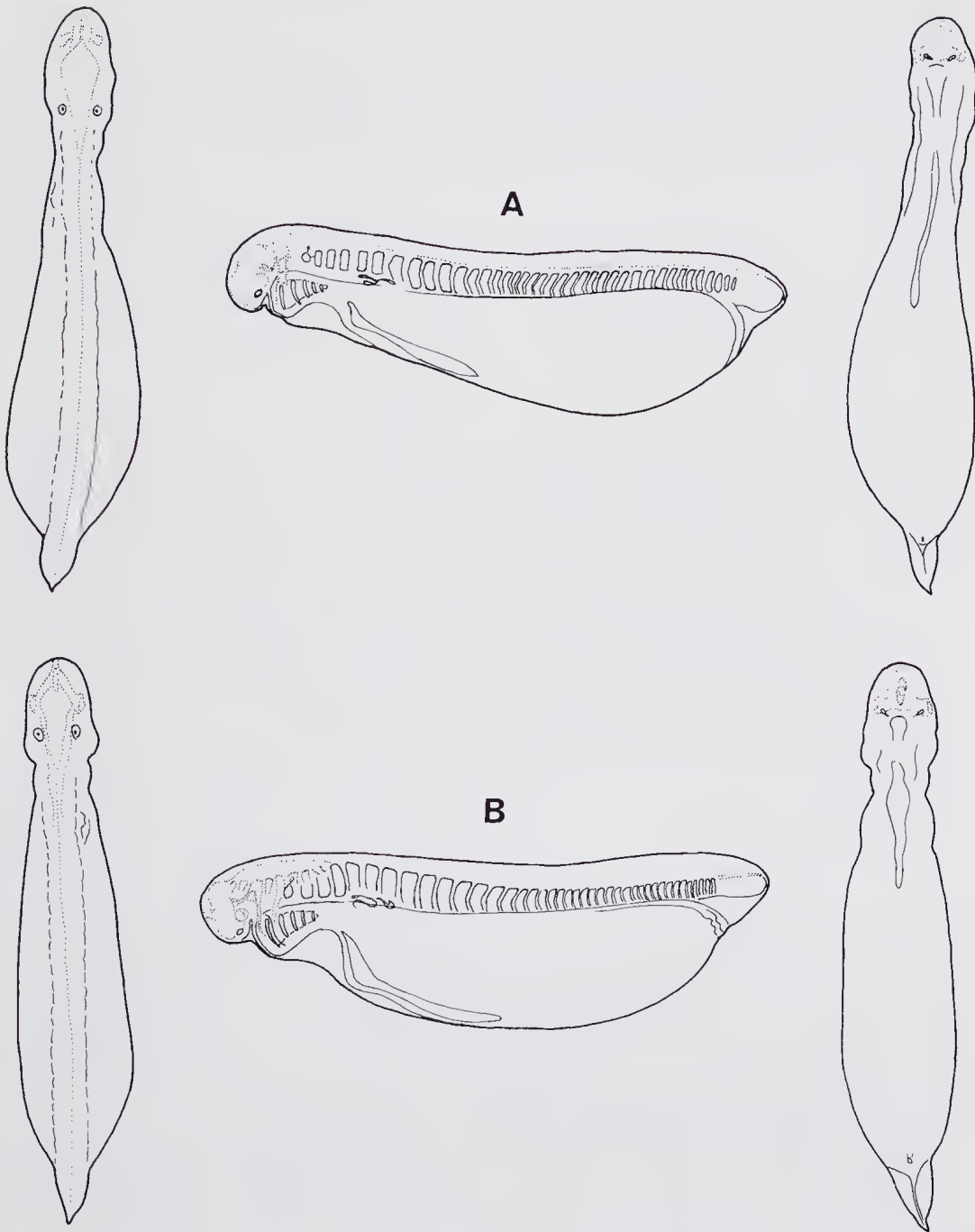


FIG. 9. Later neurulae drawn from cleared specimens. Dorsal view on the left, lateral in the centre and ventral on the right. A, stage 35, with 37 somites, less distinct pronephros showing two tubules and a duct, initial development of the upper lip, the rectum and the tail bud and continued elongation of the endodermal mass; B, stage 36, showing 40 somites and further development of the tail bud, rectum and mouth cavity and the appearance of the opercular fold. Scale line = 1 cm.

capsule with the head bent round to the anus. At stage 32, the space within the membranes starts to expand, and the embryo becomes more active. Large cracks appear in the vitelline membrane at stage 35–37 (Pl. 1B). The embryo moves more freely and soon the vitelline and inner albumen membranes break down completely and the outer albumen membrane is dissolved from within, thus enlarging the cavity in which the fish lives. The broken membranes lie in pieces at the bottom of the egg capsule.

Hatching occurs between stages 43–46 when a small hole appears in the albumen capsule and the young fish can escape.

ASSUMPTION OF THE ADULT FORM AND HABITS

The globular shape of the endodermal mass disappears at stage 31–34 as the body extends and the number of somites increases. A proper tail starts to grow at stage 36–37 (Pl. 3A). The adult form develops gradually as the fins appear and assume the adult sizes and proportions (Pl. 3E–H). There is no obvious metamorphosis.

For a long time the body of the juvenile is very thin (between stages 44 and 54), giving the animal a peculiar appearance in dorsal or ventral view. Young fish (Pl. 3G and Figs. 16 and 17) are much slimmer than adults but gradually the trunk becomes thicker and the body form of the adult appears.

Larvae of stage 52 have been observed to breathe air in laboratory aquaria.

DISCUSSION

Eggs of *Neoceratodus forsteri* have a thick albumen coat apparently like *Protopterus dolloi* (Pasteels 1962: 175) but unlike *Lepidosiren paradoxa*, which usually has no jelly (Kerr 1900: 308). Kerr suggests that this is because *L. paradoxa* eggs develop in the comparative security of a guarded nest. However *P. dolloi* eggs also develop in a nest and the function of the jelly remains unknown. The hatching process, involving breakdown of the inner membranes, also requires further elucidation.

The eggs of all three lungfish are very large compared with those of many Amphibia, and possess large amounts of yolk (Kerr 1919). The eggs of *L. paradoxa* are spherical, 6.5–7 mm in diameter with pink yolk and a white germinal cap of more finely divided yolk. There is no dark pigment in the eggs (Kerr 1900: 308). *P. annectens* lays smaller eggs, also spherical, with a diameter of 3.5–4 mm which have green yolk and

a pink epiblastic pole (Budgett 1901). Pasteels (1962: 175) describes the eggs of *P. dolloi* as 4 mm in diameter, yellowish cream in colour and entirely without pigment. The animal pole of living eggs has a rosy tint and the vitelline mass is greenish. Eggs of *N. forsteri* are the smallest, 3–3.5 mm in diameter, hemispherical in shape with green or brown yolk granules. They have granules of dark pigment, usually in the uppermost parts of the egg. Possibly this protects the eggs as they often develop in situations exposed to light, an adaptation which is not necessary for the eggs of other lungfish which develop in dark underwater burrows.

In eggs of *N. forsteri*, pigment is scattered sparsely over most of the egg surface and is dense only in a small area. Cleavage is usually initiated in this area, no matter where it is on the egg i.e. round or flat surface. Many abnormalities in cleavage can be traced to the unusual position of pigment on the egg. The animal pole appears not to be that part of the egg which is flat and floats uppermost, but is the region that has the bulk of egg pigment; the vegetal pole is the part of the egg most free of these granules. Usually, the pigment and therefore the animal pole is on the flat surface which is uppermost.

Various external features that are prominent in some newly fertilised eggs may be compared with those in some amphibians. For example the dark spot may correspond to the point of sperm entry and the pale area mark the position of the oocyte or zygote nucleus. There is, however, little evidence to support these ideas. The dark spot is found in some uncleaved eggs and soon disappears. The pale area is shared out among cleavage products in eggs which possess one, but their nuclei are not superficial in position either at the beginning of cleavage or later, while the pale area persists. Nuclei lie well below the surface between the small peripheral yolk granules and the large central yolk granules.

It is still possible that the pale area corresponds to the position of a superficial oocyte nucleus, but if so it is difficult to explain the persistence of the pale area after the nucleus moves inwards. Alternatively the pale area may mark the position of sperm entry and function like the grey crescent of certain amphibian eggs. If this is true the pale area should be found in a position on the surface of the blastula equivalent to its position in the uncleaved egg. However it is split up and shared out to cleavage products and cannot be traced after stages 3 or 4.

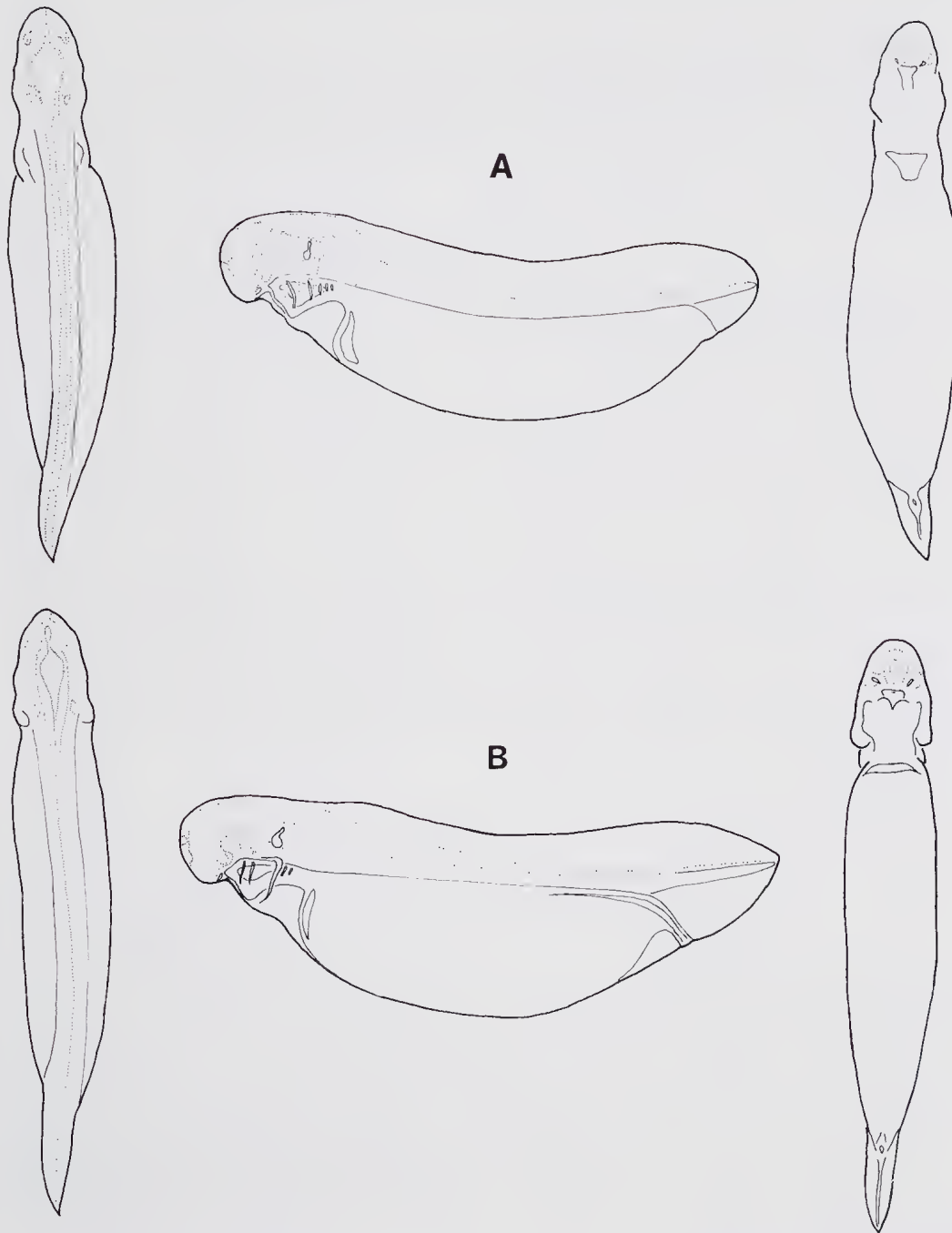


FIG. 10. Drawings of cleared specimens showing the development of the median fins. Dorsal view on the left, lateral in the centre and ventral on the right. A, stage 37, showing the development of the dorsal fin and the ventral post-anal fin as the body extends, and the growth of the opercular fold and upper lip (involving the nares); B, stage 38, with the initial development of the ventral pre-anal fin, dorsal fin reaching the back of the head, growth of the opercular fold to cover the anterior gill slits and the appearance of the lower lip. Scale line = 1 cm.

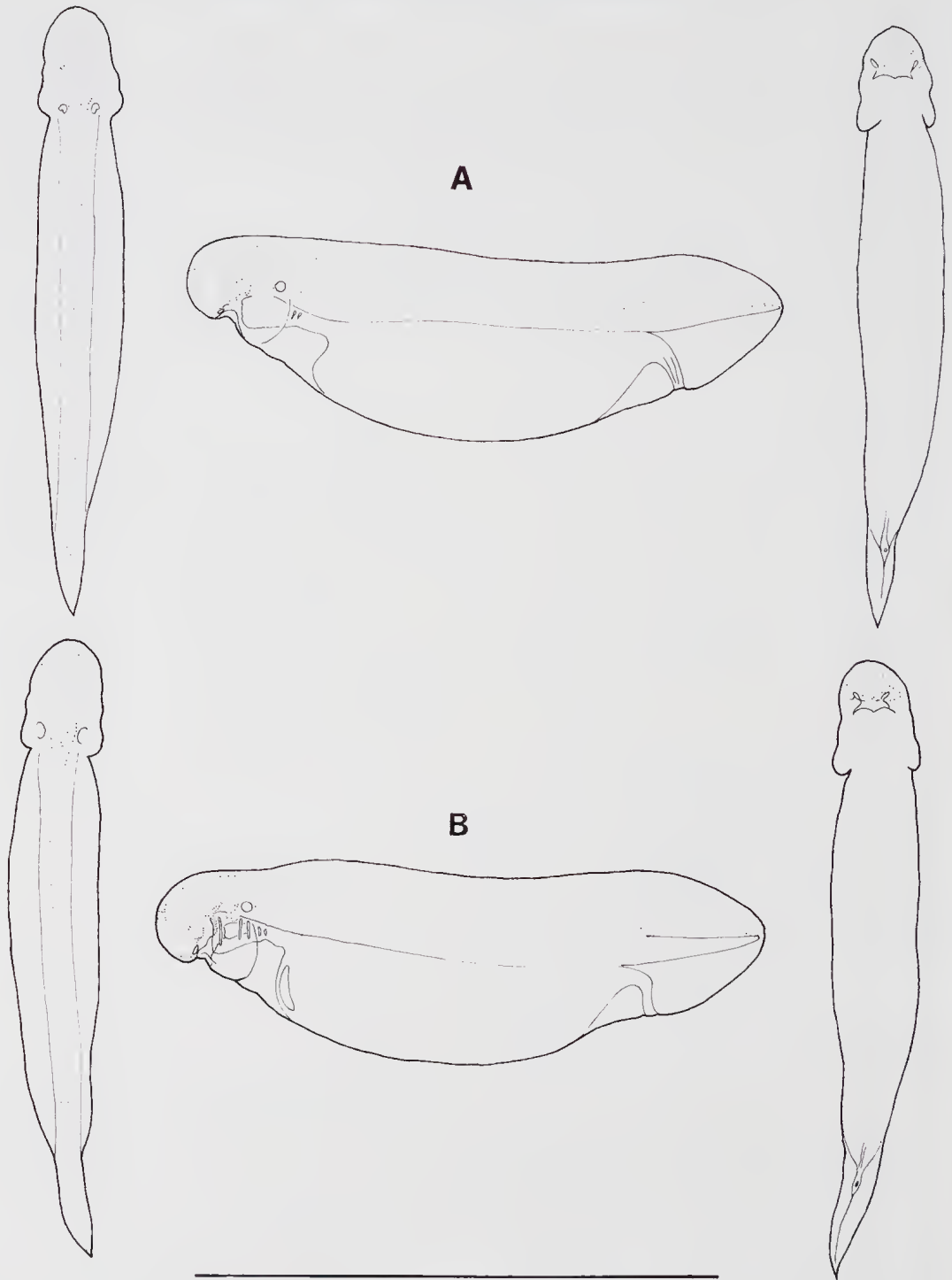


FIG. 11. Drawings of cleared specimens showing growth of the median fins. Dorsal view on the left, lateral in the centre, and ventral on the right. A, stage 39, operculum covering anterior 3 gill slits, forward growth of the lower lip, continuing involvement of the nares in the formation of the upper lip, development of the rectum and increasing length of the tail; B, stage 40, showing primordium of the trunk lateral line behind the operculum. Increasing density of the melanophores in the skin makes it less rewarding to examine cleared specimens. Scale line = 1 cm.

Abnormalities of cleavage in *N. forsteri* are common and do not always prevent subsequent development. Obviously some types of abnormal cleavage are more detrimental to development than others but the number of eggs of unusual pattern available was not great enough to compare individual abnormalities with normal eggs.

Many of the unusual patterns of cleavage may be directly related to the region of the egg in which the pigment is located, but some are more difficult to explain, for example a three cornered division or latitudinal first cleavage. It would be interesting to know if abnormal patterns in early cleavage are found in other lungfishes or in Amphibia, with similar frequencies and as little effect on later development. Miller (1923) found some irregular patterns in *L. paradoxa*. Unusual cleavage patterns, whatever their cause, disappear when the blastocoel expands and the egg prepares for gastrulation.

The blastocoel increases in size until the egg fills the whole intravitelline space and is no longer able to rotate. It is possible that the egg needs the mechanical support of the vitelline membrane for normal gastrulation movements to occur. Previous descriptions of gastrulation in *N. forsteri* (Semon 1893, 1901a) have not shown details of the change from a crescentic dorsal lip lying across the egg (midgastrula) to a dorsoventrally orientated slit (early neurula).

Examination of a complete series of fixed and living embryos in the process of gastrulation suggests that the mode of formation of the blastopore is similar in *N. forsteri*, *P. dolloi* and Amphibia, particularly Urodela (Anderson 1943; Weisz 1945; Eakin 1947; Rugh 1962). In the Queensland lungfish, the curved dorsal lip appears early and there is little external change for a long time (Stages 12–13). Wide lateral lips form next. The ventral lip normally develops very late after the drawing together of the lateral lips has almost converted the blastopore into a dorsoventral slit (Stages 14–16). These stages are transient. A common variation, which does not hamper future development, results in a large yolk plug like that of an amphibian embryo (for example, a Stage 12 embryo of *Triturus pyrrhogaster*, Anderson 1943). In addition, there is a close external similarity between *N. forsteri* and *T. pyrrhogaster* gastrulation in later stages.

In *P. dolloi*, the blastopore is at first a slit, then crescentic, as in *N. forsteri*. Later, after the invagination of the yolky cells, it is ring-shaped with the dorsal and ventral lips in apposition, lying transversely across the egg (Pasteels 1962: 177).

The condition in *L. paradoxa* (Kerr 1900, plate 8, figs. 12, 13 and 14 and plate 9, fig. 15) and in *P. annectens* (Budgett 1901 plate 10) appears to be similar. Differences in gastrulation in *N. forsteri*, *P. dolloi*, *P. annectens*, *L. paradoxa* and amphibians appear to be minor, involving the shape of the blastopore, and the time taken for the yolky cells to be invaginated and for the ventral lip to appear.

Changes in the shape of the blastopore of fixed specimens of successive stages probably reflect no more than the plasticity of the blastoporal lips in the living egg, i.e. moving in and out and sometimes joining to produce two openings, one within and one outside the neural folds. The blastopore ultimately gives rise to the anus, as in *L. paradoxa* (Kerr 1900: 313), and it is linked for a long time to the nerve cord via the neurenteric canal, a feature absent in *L. paradoxa* (Kerr 1900: 312).

It is curious that the globular portion of the endodermal cell mass is positioned under the head region in *L. paradoxa* and *P. annectens* and more posteriorly in *N. forsteri* (Kerr 1909).

The neural rudiment in *L. paradoxa*, as in lampreys and teleostomatous fishes, forms by the downgrowth of the medullary plate as a solid keel of tissue (Kerr 1902: 22; 1919: 83) and the central cavity characteristic of vertebrate nervous systems develops later either by the appearance of a fine intercellular split or by the breakdown of cells along the axis. However, traces of the formation of the spinal cord by the meeting of two folds may be found (Kerr, 1902: 23 and Pl. 4). The nervous system of *N. forsteri* is always tubular (Greil 1908).

The primary vesicles of the brain in *N. forsteri* appear before the neural folds have closed, and in *L. paradoxa* and *P. annectens* they do not develop until after the folds close (Kerr 1909), but this is not a fundamental difference. The presence of a distinct transverse crest linking the anterior neural folds in embryos of stage 20–23 in *N. forsteri* is reminiscent of urodele development (Anderson 1943).

Many aspects of early development in *N. forsteri* are similar to those of amphibian embryos e.g. the arrangement of cleavage furrows, formation of the blastopore, appearance of a transverse crest in the anterior neural plate, development of the nerve cord as a tube and the shape of the embryos. However such similarities are common among diverse lower vertebrate groups. Parallels may equally well be drawn between the arrangement of cleavage furrows in

TABLE 5: CHARACTERISTICS OF STAGES 23-28

Stages	Blastopore /anus	Nervous System	Sense Organs	Somites	Pronephros	Stage (Semon 1893)	Stage (Kerr 1909) <i>L. P. paradoxo annectens</i>
23 (Fig. 6C)	2 openings, upper within neural folds, lower outside	folds raised almost as far as the blastopore and closer together, no furrow in centre, transverse crest sinks further	first indication of opticoele in cleared specimens	5; form at anterior and at posterior end of mesoderm block	—	24	18 XVIII
24 (Fig. 6D)	single, upper part within neural folds	transverse crest gone, folds closed anteriorly, raised around blastopore	opticoele distinct in cleared specimens	7	—	26	20 —
25 (Fig. 6E)	single, not continuous with neural canal	neural rudiment hollow folds still open at posterior extremity	opticoele develops, auditory placode shows externally as pigmented pit	10-11, new somites form only at posterior end from this stage	—	27	— XX
26 (Fig. 7A, Plate 2F)	continuous with neural canal	folds entirely covered in epithelium	auditory placode sinks inwards	13	—	28	21 —
27 (Fig. 7B)	blastopore (anus) continuous with gut cavity and neural canal	fore-brain separates into two parts	opticoele more distinct from brain	15-17	visible externally as small bulge behind auditory vesicle	29	— XXI
28 (Fig. 7C)	blastopore (anus) continuous with gut cavity and neural canal	auditory vesicle close to metencephalon	auditory vesicle close to metencephalon	18-20	—	—	23 —

Acipenser and in *N. forsteri* (Kerr 1919: 24) or between gastrulation and the general shape of embryo in lungfish and lampreys (Balfour 1881: 73 and Kerr 1919: 38).

The appearance of a pre-anal ventral fin at stage 38 and its growth and subsequent regression by stage 53 is a curious feature for which no parallel can be found in fossil or living Dipnoi, though some fossil specimens seem to have had separate post-anal ventral fins (*Dipterus valencienesi*, Forster-Cooper 1937, *Fleurantia denticulata*, Graham-Smith and Westoll 1937 and *Phaneropleuron andersoni*, Dollo 1895). Certain young Amphibia possess traces of a pre-anal fin for example Urodela like *Ambystoma punctatum* (stage 40) and *Taricha torosa* (stage 39) (Rugh 1962) and Anura like *Hyla regilla* (stages 23 and 24) (Eakin 1974) and *Xenopus laevis* (stages 20–23) (Weisz 1945). The transient appearance of a double flap of skin in front of the anus in the mid-ventral line is not a firm basis for suggesting links between lungfish and amphibia but it is a further point of similarity in the external features of their young forms.

Close similarity in external appearance between *N. forsteri* and amphibian embryos ends at stage 42 when the pectoral fins appear and specifically dipnoan characteristics become increasingly recognisable.

The times of appearance of the pectoral fins at stage 42 and the pelvic fins at stage 48 in the present material do not agree with those given by Semon (1893) who found pectoral fins at his stage 45 and pelvic at his stage 47. This may reflect a genetic difference in the embryos or perhaps variation in the conditions under which the animals were reared. Also at a stage in the present series corresponding to Semon's stage 45, in which he described scales, no scales were found. The primordia of scales in this material were not apparent externally until stage 53 and were not easy to see until stage 54.

Differences in the external features of developing *N. forsteri* from the Brisbane River and Enoggera Reservoir are minor — the shape of the cleaving egg and the times of appearance of the pectoral and pelvic fins and the sensory lines of the head.

Juveniles of all three genera of lungfish begin to breathe air at approximately the same stage of development; *P. annectens* when it is 25 mm long (Johnels and Svensson 1955), *L. paradoxa* at 30 mm (Kerr 1900) and *N. forsteri* at 25 mm (stage 52). Young fish also respond to light in a similar way (Kerr 1900), though *N. forsteri* juveniles lose

this ability when the skin pigment becomes uniformly dark over the dorsal surface.

Unlike most members of the Amphibia, there is no obvious metamorphosis in *N. forsteri*. The animal gradually assumes an adult form without sudden changes.

The number of stages in the present work is seven more than in Semon's account (1893) but five of these are older than his latest stage. There are also more stages than given by Kerr (1909). Older specimens are included in the present work as well as more cleavage and gastrulation stages. It is often important in a living embryo to be able to estimate as precisely as possible the stage of development from external appearances. These features have been emphasised in this account.

ACKNOWLEDGEMENTS

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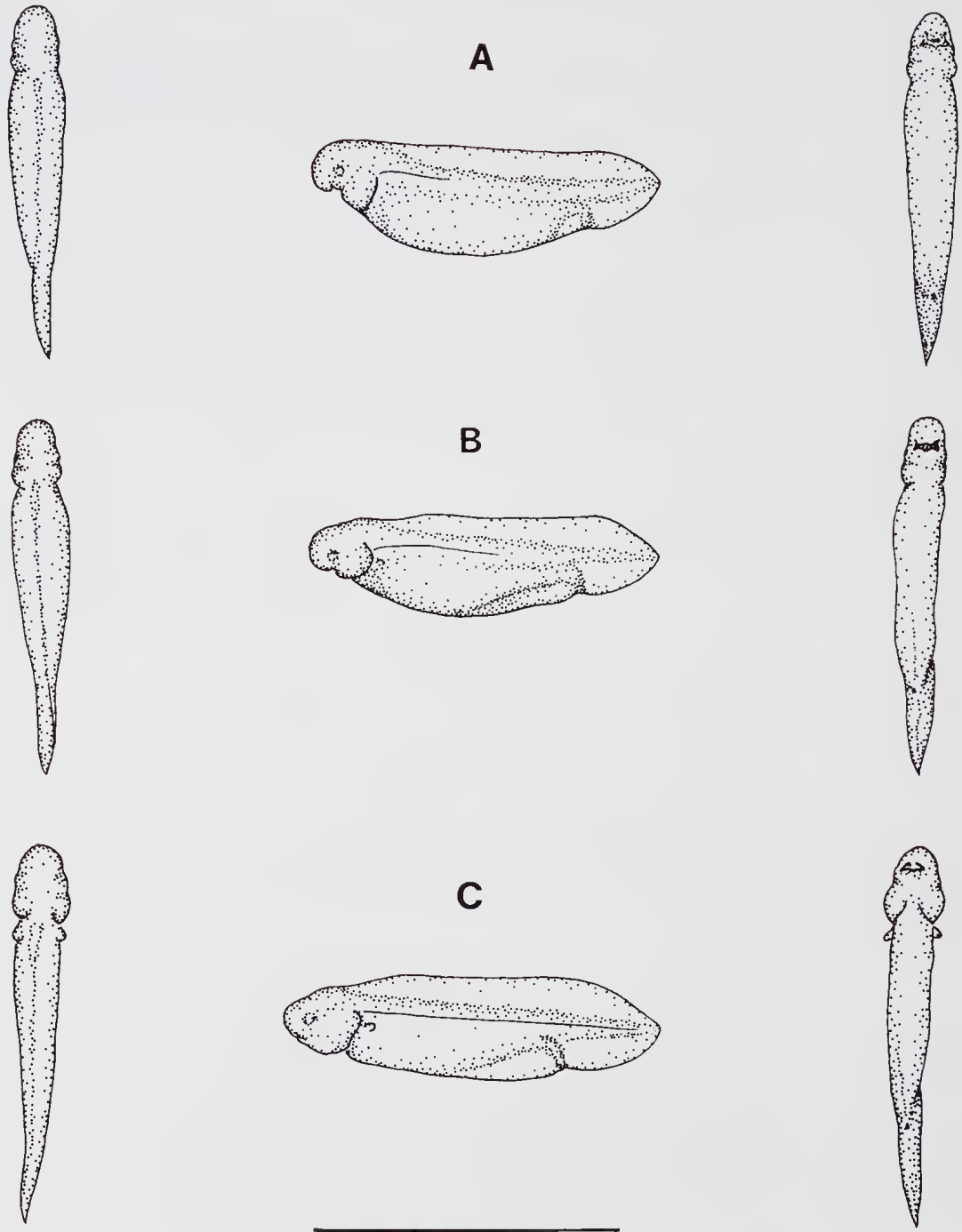


FIG. 12. Drawings of the embryos removed from the egg membranes before fixation showing the growth of the trunk lateral line and the appearance of the pectoral fin. Dorsal view on the left, lateral in the centre and ventral on the right. A, stage 41, showing operculum covering all the gill slits, forward growth of the lower lip and lateral line extending one-third of the way down the body; B, stage 42, in which the lateral line extends halfway down the body and the primordium of the pectoral fin appears; C, stage 43, with a longer lateral line stopping short of the tip of the tail, larger pectoral fin, and nares still visible in the upper lip. Scale line = 1 cm.

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TABLE 6: CHARACTERISTICS OF STAGE 29-33

Stage	External Features	Nervous System	Sense Organs	Somites	Pronephros	Stage (Semon 1893)	Stage (Kerr 1909) <i>L. paradoxo annectens</i>
29 (Fig. 7D)	expanded anterior cavity, very narrow canal reaches anus; first external sign of mouth	neurenteric canal present, dorsal root ganglia develop	auditory vesicle close to hind brain	24, oblong shape	visible externally behind auditory vesicle	—	XXIII
30 (Fig. 7E, Plate 2G)	2 visceral clefts endodermal mass globular	thin roof of hind brain shows	eye visible externally, auditory vesicle linked with auditory nerve, first sign of olfactory placodes anterior to mouth region	26	in cleared specimens associated with post-otic somites 4, 5, and 6	30	25
31 (Fig. 8A)	mouth is a little deeper	expansion and forward growth of brain	olfactory placodes sink	30	in cleared specimens associated with post-otic somites 4, 5, and 6	32	XXV
32 (Fig. 8B, Plate 2H)	as long axis of body begins to grow, endodermal mass less globular	expansion and forward growth of brain	olfactory capsule open to exterior	33	compact organ, 2 nephrotomes and duct	34	—
33 (Fig. 8C, Plate 2I)	liver diverticulum forms, 2 more visceral clefts appear mouth is a triangular area of pale endoderm showing through epithelium	expansion and forward growth of brain	—	35 V-shaped	duct grows posteriorly	36	27 XXVII

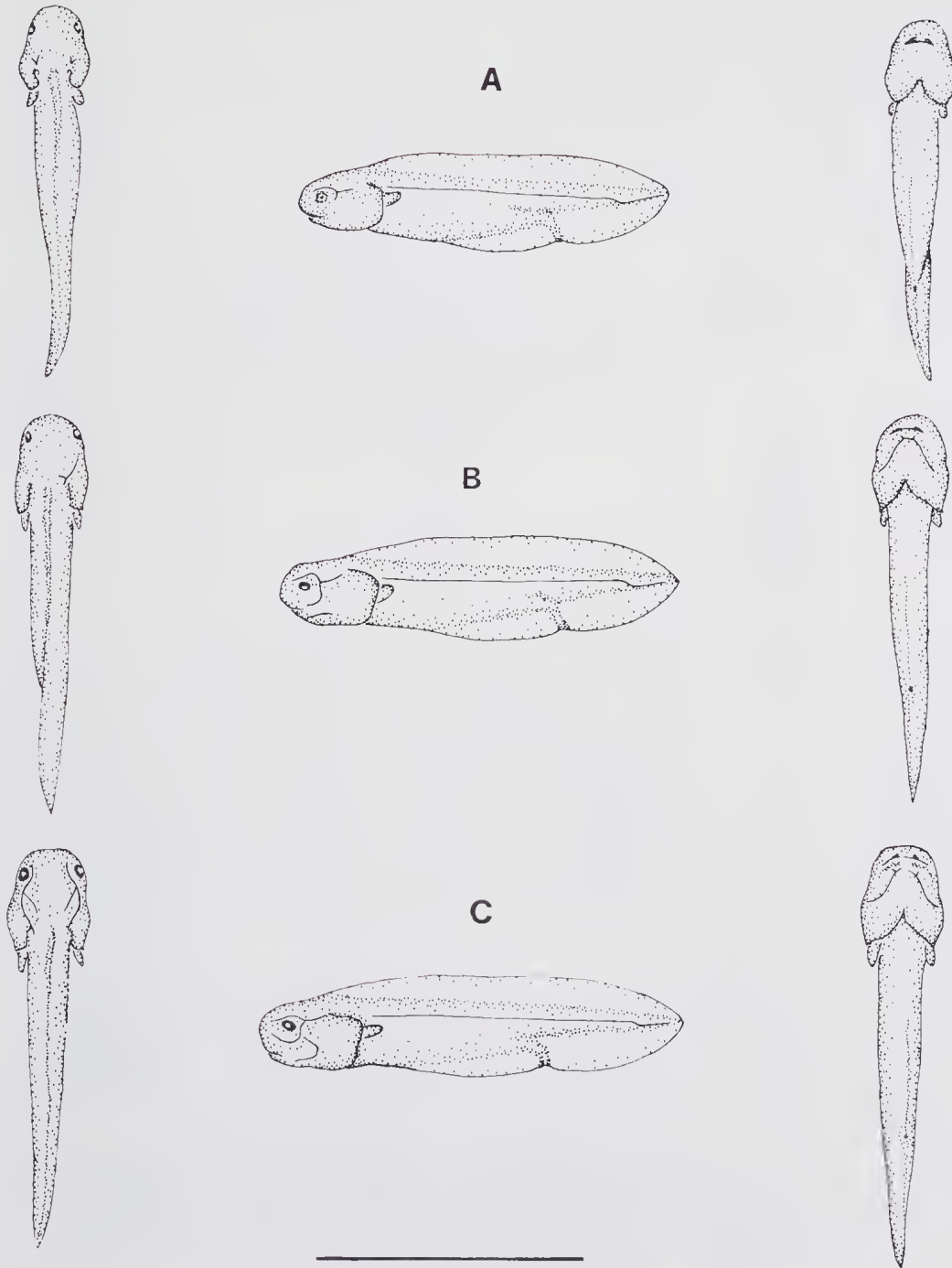


FIG. 13. Development of the pectoral fin and appearance of the sensory lines of the head. Dorsal view on the left, lateral in the centre, and ventral on the right. A, stage 44, in which the pre-anal ventral fin is growing forwards and the dorsal reaches the back of the head. The lateral line of the trunk reaches the tail tip. Infra- and supra-orbital head lines are present; B, stage 45, showing two more sensory head lines dorsally, the temporal and posterior lines, and one ventrally, the mandibular; yolk is still present in the gut and much of the pectoral fin is overgrown by the operculum; C, stage 46, with links forming between the sensory head lines, jugal line and gular pits beginning to form behind the lower lip. The mouth has shifted anteriorly. Scale line = 1 cm.

TABLE 7: CHARACTERISTICS OF STAGES 34-38

Stage	Gut	Medial Fins	Chromatophores	Somites	Pronephros	Stage (Semon 1893)	Stage (Kerr 1909) <i>L. paradoxo</i> <i>P. annectens</i>
34 (Fig. 8D)	neurenteric canal still present, mouth grows a little deeper, 5 gill clefts present	tail bud		36	Pronephros prominent externally	37	—
35 (Fig. A, Plate 2I)	neurenteric canal disappears, upper lip begins to develop, as tail bud straightens and embryo grows longer the globular shape of the yolk is lost	tail bud extends and straightens	small numbers of melanophores in epidermis of dorsal surface and around lens	37	less prominent	38	28 XXVIII
36 (Fig. 9B)	rectum begins to develop and operculum appears	dorsal fin $\frac{1}{2}$ of length of trunk	more melanophores	39	no longer prominent externally	39	— XXIX
37 (Fig. 10A, Plate 3A)	developing upper lip involves nerves, 'platelet' stage of endoderm cells second cleft covered	dorsal fin $\frac{1}{2}$ of length of trunk small ventral post-anal fin	melanophores spread ventrally	39		—	30 XXXI
38 (Fig. 10B)	operculum covers 3rd cleft, mouth cavity deeper, lower lip starts to form	dorsal fin reaches back of head, post-anal ventral fin grows, small pre-anal ventral fin appears	melanophores increase			41	— XXXII

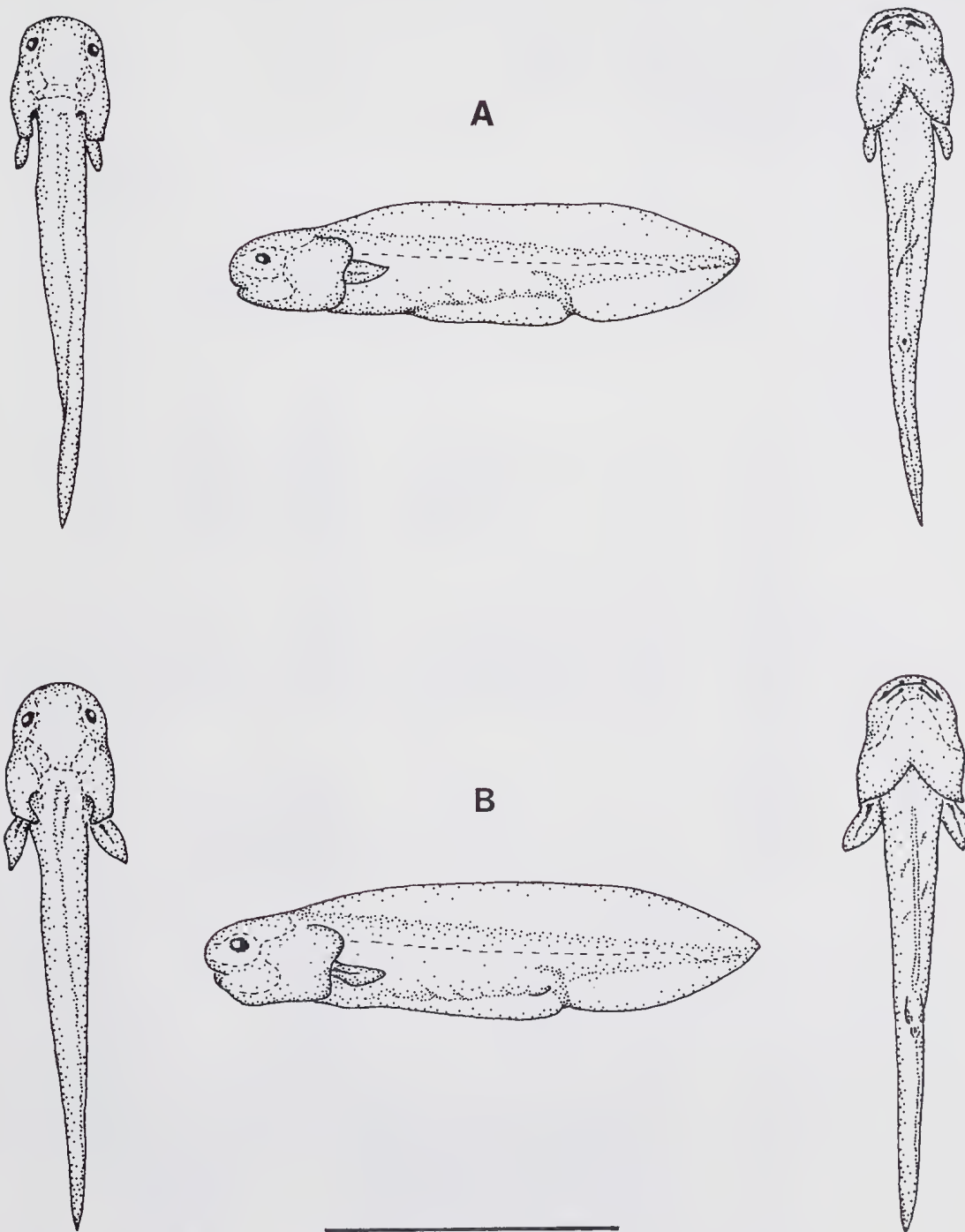


FIG. 14. Drawings of fixed fish showing the appearance of the spiral valve and of the pelvic fin. Dorsal view on the left, lateral in the centre, and ventral on the right. A, stage 47, in which the spiral valve shows clearly as the last of the yolk disappears, the skeleton of the pectoral fin shows through the skin, the sensory lines break up into a series of pits and the supratemporal cross commissure appears; B, stage 48, in which the primordium of the pelvic fin appears in lake eggs, gular pits are well-formed and the pre-anal ventral fin reaches the level of the opercular folds in the mid-ventral line. s. — spiral valve. Scale line = 1 cm.

TABLE 8: CHARACTERISTICS OF STAGE 39-43

Stage	External Features	Medial Fins	Paired Fins	Chromatophores	Lateral Line	Stage (Semon 1893)	Stage (Kerr 1909) <i>L. paradoxa</i> <i>P. annectens</i>
39 (Fig. 11A)	endoderm cells packed into blocks, operculum larger			melanophores increase		42	31
40 (Fig. 11B)	operculum covers 4 slits, lower lip distinct	dorsal and ventral fins grow		melanophores increase		43	XXXIV
41 (Fig. 12A)	operculum covers all posterior gill slits, mouth cavity joins foregut, lower lip grows forwards			melanophores increase	lateral line of body 1/3 way along trunk	—	—
42 (Fig. 12B, Plate 3B)		pre-anal fin grows forward mid-ventrally	Primordium of pectoral fin appears behind operculum	melanophores increase	lateral line of body 1/3 way along trunk	44	32
43 (Fig. 12C)	opercular folds almost meet midventrally; yolk content of cells reduced		pectoral fin grows, has pigment cells	red chromatophores present dorsally	lateral line almost reaches tail tip	—	—

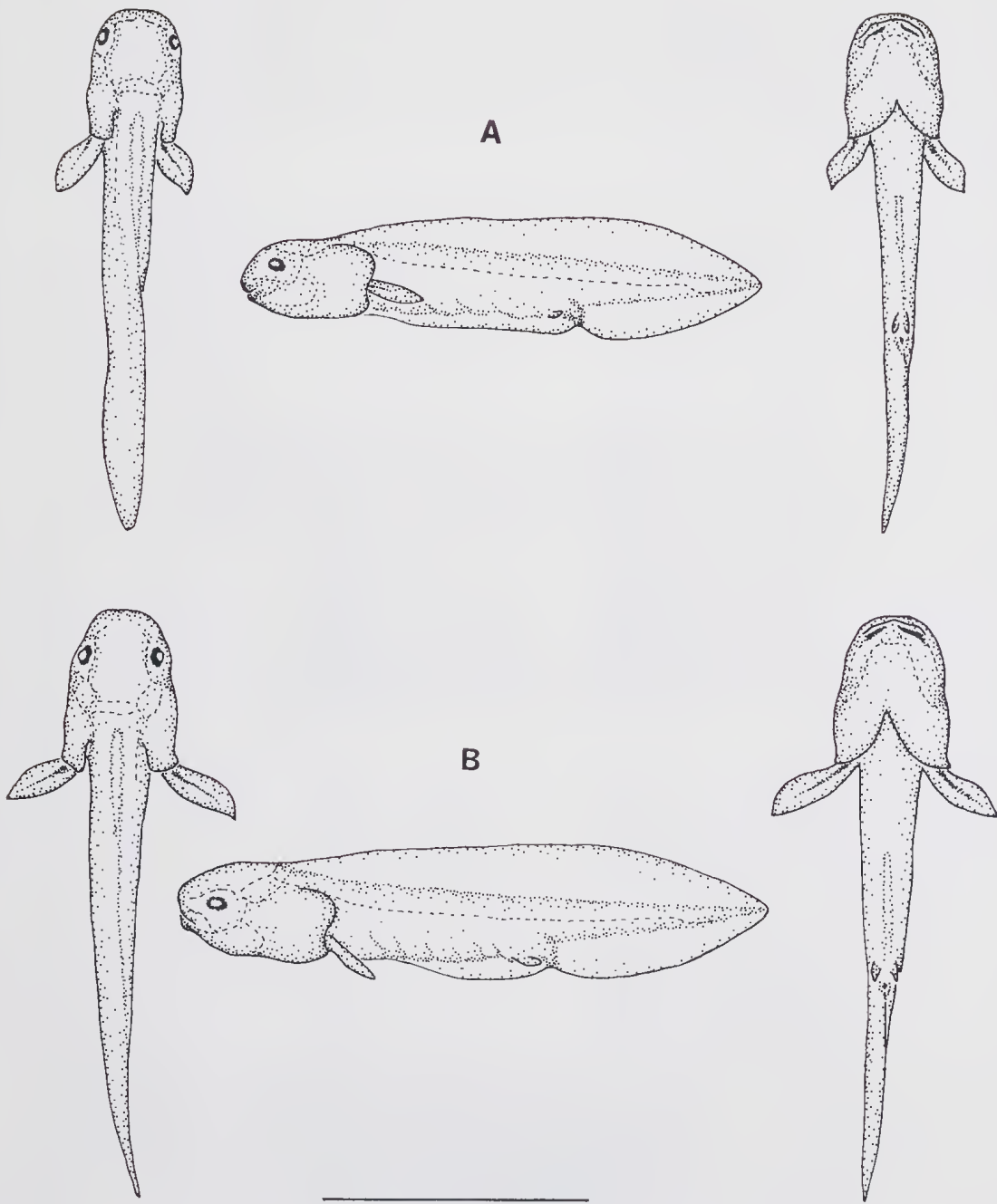


FIG. 15. Drawings of fixed fish showing the regression of the pre-anal ventral fin and growth of the pelvic fins. Dorsal view on the left, lateral in the centre, and ventral on the right. A, stage 49, with an additional line of sensory pits, the oral line, running along the lower lip, some regression in the ventral pre-anal fin and growth of the pelvic fin; B, stage 50, showing further regression of the pre-anal ventral fin and extension of the paired fins. Scale line = 1 cm.

TABLE 9: CHARACTERISTICS OF STAGE 44-49

Stage	Gut	Medial Fins	Paired Fins	Chromatophores	Lateral Line System	Stage (Semon 1893)	Stage (Kerr 1909) <i>L. P.</i> <i>paradoxa annectens</i>
44 (Fig. 13A, Plate 3C)	quantity of yolk in endoderm cells reduced, lower lip grows forwards and almost meets upper lip, anterior shift of mouth starts, nares are visible within upper lip, opercular folds meet in mid-ventral line	pre-anal fin extends half way along yolk cell mass	pectoral fin grows	many pigment cells of both types; an- imal changes colour according to light	head lines indicated body line bends slightly just before reaching tail	45	—
45 (Fig. 13B)	lower lip meets upper		pectoral fin partly covered by operculum	many pigment cells of both types; an- imal changes colour according to light	in side view, tempor- al, supra- and infra- orbital and body lines are present; in dorsal view temporal and posterior head lines, and in ventral view the mandibular line	46	—

TABLE 9: (Continued)

Stage	Gut	Medial Fins	Paired Fins	Chromatophores	Lateral Line System	Stage (Semon 1893)	Stage (Kerr 1909) <i>L. paradoxa</i> <i>P. annectens</i>
46 (Fig. 13C)	mouth shifts further anteriorly, indications of gular pits in lower lip	pre-anal fin extends further forwards		many pigment cells of both types; animal changes colour according to light	jugal line present	—	34
47 (Fig. 14A, Plate 3D)	yolk in endoderm cells sufficiently reduced to show spiral valve of gut, gular pits develop on either side of lower lip		axial skeleton of pectoral fin appears	many pigment cells of both types; animal changes colour according to light	supra-temporal cross commissure appears, lines break up into pits	—	—
48 (Fig. 14B, Plate 3E)	yolk reduced, spiral valve well developed	pre-anal fin reaches back of opercular folds	primordium of pelvic fin appears anterior to anus	many pigment cells of both types; animal changes colour according to light		47	35
49 (Fig. 15A, Plate 3F)	yolk gone	pre-anal fin starts to regress, fin rays present in dorsal and post-anal ventral fin	pectoral fin large, mostly free of operculum, pelvic fin has pigment cells	many pigment cells of both types; animal changes colour according to light	oral line present	—	XXXVI

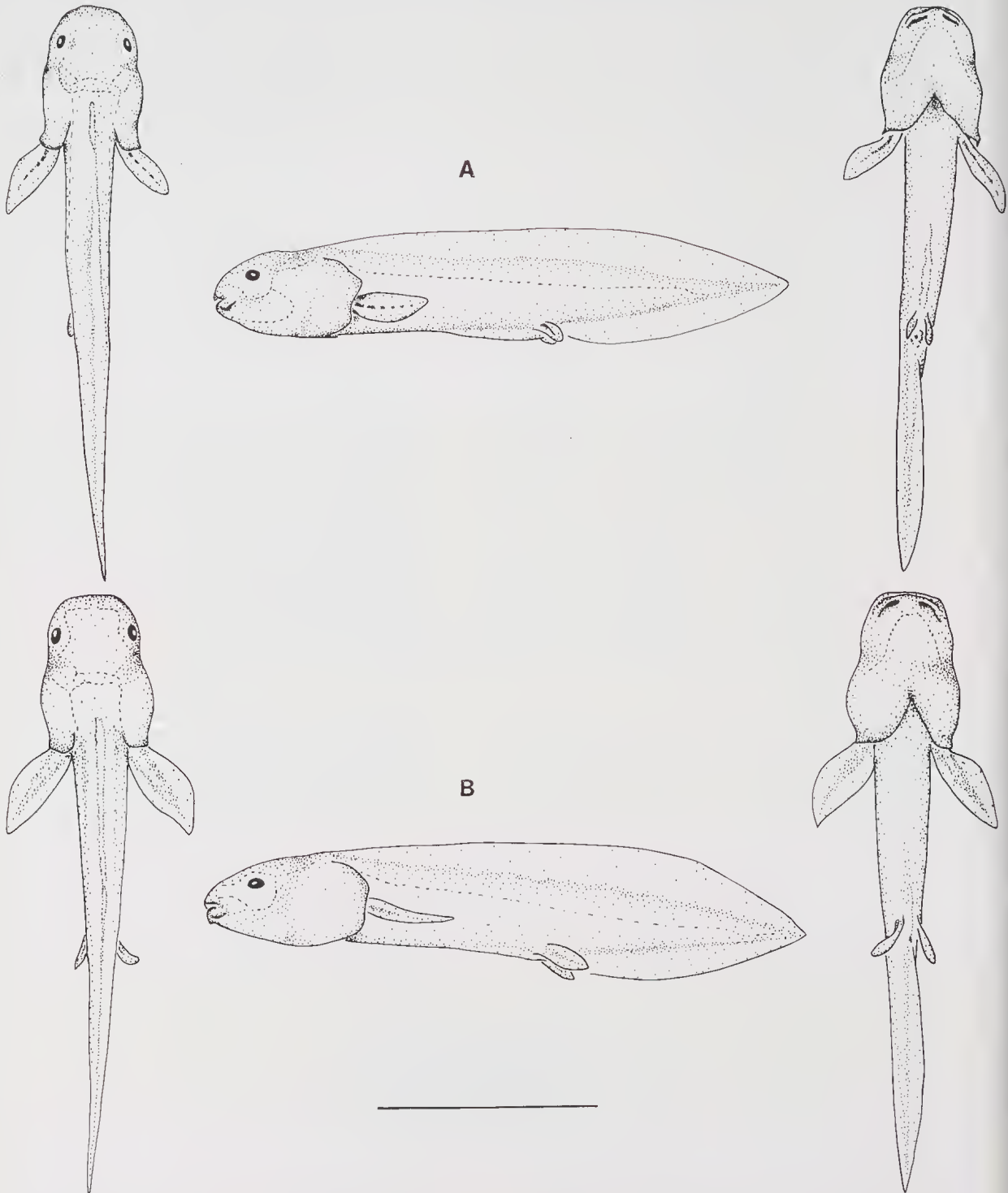


FIG. 16. Drawings of fixed fish. Dorsal on the left, lateral in the centre, and ventral on the right. A, stage 51, with a broad head, narrow body, nearly terminal mouth and showing regression of the ventral pre-anal fin; B, stage 52, the ventral pre-anal fin is almost gone and the head and paired fins are large in relation to the body. The mouth is terminal at this stage. Scale line = 1 cm.

TABLE 10: CHARACTERISTICS OF STAGES 50-55

Stage	External Features	Mouth	Nares	Medial Fins	Paired Fins	Chromatophores	Lateral Line System
50 (Fig. 15B)	head broad, body narrow (cf. stage 48 Semon 1893)		anterior portion of anterior nares show with- in upper lip	pre-anal fin continues to regress		pigment cells of both types are present all over the body, larva shows marked response to light	as stage 49 but less easy to see
51 (Fig. 16A, Plate 3G)		terminal in position	nares do not show	pre-anal fin extends less than half way to operculum	axial skeleton present in pelvic fin	pigment cells of both types are present all over the body, larva shows marked response to light	
52 (Fig. 16B)	scale primordia appear	terminal in position		pre-anal fin nearly gone			
53 (Fig. 17A)	body begins to thicken (cf. <i>L. paradoxus</i> st. 36, Kerr 1909)			no pre-anal fin	pelvic fin large, both fins used to support body when feeding	pigment cells of both types are present all over the body, larva shows marked response to light	
54 (Fig. 17B)	scales distinct	no longer terminal	in ventral view anterior nares show	medial dorsal fin does not reach head	pelvic fin large, both fins used to support body when feeding	heavily pigmented dorsally, belly pale, red chromatophores not obvious, response to light lost.	hard to distinguish on head
55 (Plate 3H)	scales of adult pattern, body of adult proportions	not terminal, lower jaw narrower than upper, fits within, cf. adult	in ventral view anterior nares show	medial dorsal fin ends mid-dorsally cf. adult	pelvic fin large, both fins used to support body when feeding	spots of intense dark brown pigment appear on the dorsal fin	hard to distinguish on head

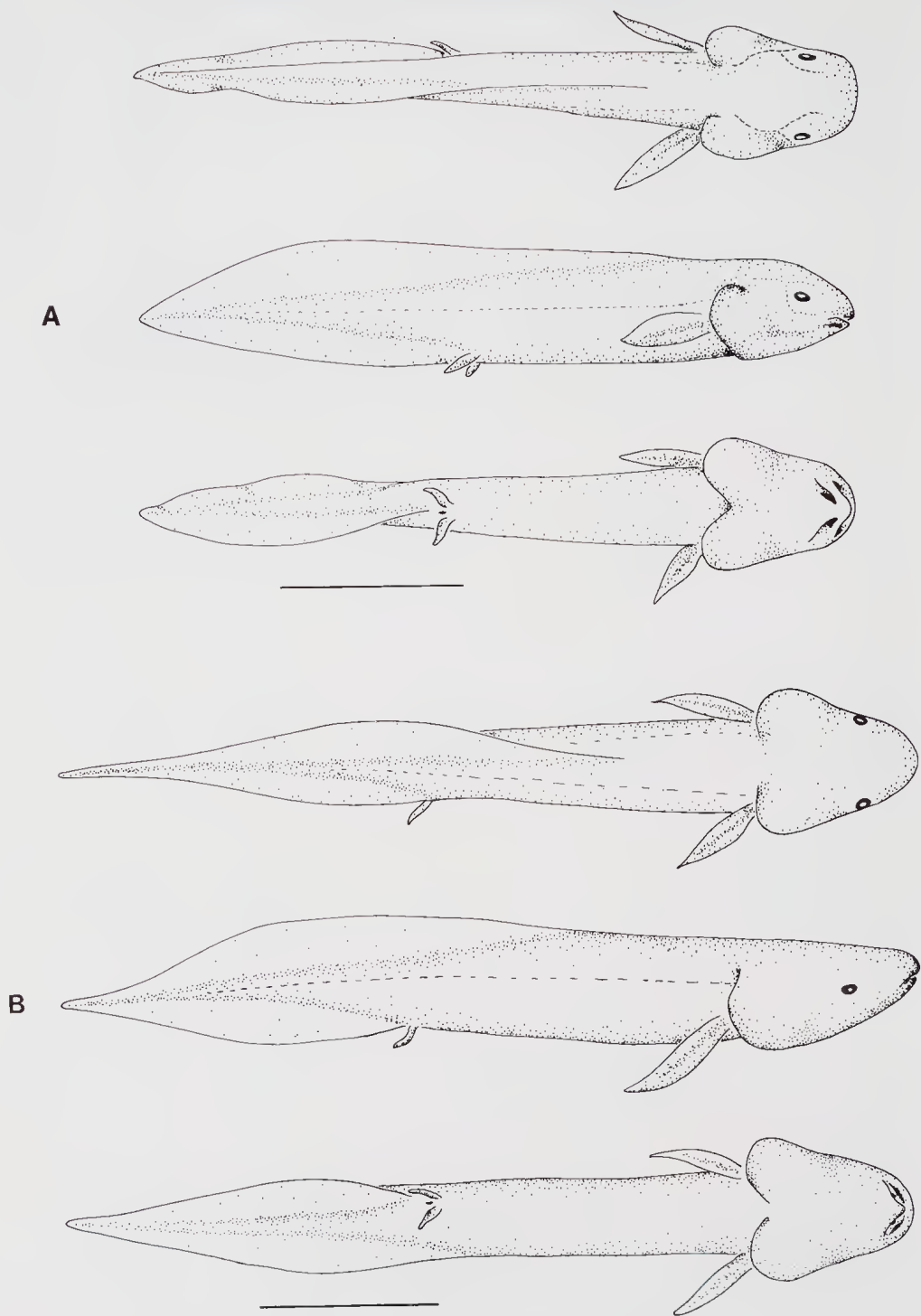


FIG. 17. Drawings of fixed fish showing the gradual assumption of the adult form. Upper is dorsal, lateral in the centre and lower is ventral. A, stage 53, showing thickening body, disappearance of the ventral pre-anal fin and initial regression of the dorsal fin. The mouth is not terminal; B, stage 54, showing body proportions similar to those of the adult, dorsal fin extending forwards as far as the middle of the trunk and the sub-terminal mouth with the anterior nares showing inside the upper lip. Scale line = 1 cm.

PLATE I.

EGGS AND EMBRYOS OF *NEOCERATODUS FORSTERI*

A, B within the membranes, C–I a sequence of fixed eggs removed from the membranes showing normal cleavage and blastula formation, J–L fixed eggs removed from the membranes showing abnormal cleavage patterns. A, A photograph of the flat surface of a living uncleaved egg of stage 1 showing the inner membranes, diffuse pigment, dark spot and pale area; B, a fixed embryo of stage 35 showing the outer albumen membranes, expanded intravitelline space and the broken inner membranes; C, the flat surface of a normal egg, prior to first cleavage, without pale area or dark spot and with the pigment confined to the flat surface; D, a side view of an egg at stage 3 showing the hemispherical shape and cleavage furrows, and the unequal distribution of pigment and pale area to the cleavage products; E, the flat surface of an egg at stage 4, showing three cleavage furrows bisecting the cells produced by the previous division and one furrow cutting across the cell; F, an egg at stage 5 photographed obliquely to show part of the side and part of the flat surface; there is no trace of the pale area and the hemispherical shape is less obvious as the segmentation cavity expands; G, an egg at stage 7 showing most of the unpigmented convex surface and some of the pigmented cells of the previously flat surface; cells of the convex surface are flattened against the vitelline membrane in the living egg (pavement cells) and the smaller pigmented cells project into the intravitelline space; H, a large-celled blastula of stage 8 showing the pigmented region; cell outlines are indistinct as cell division is in progress; I, a blastula at stage 9, with pigment confined to the smaller cells; the segmentation cavity is further enlarged and the hemispherical shape has been obliterated; J, the convex surface of an egg at stage 2 with pigment abnormally positioned over one third of the convex surface and an undivided pale area; K, an egg of normal pigment pattern in which abnormally positioned cleavage furrows are developing (cf. Semon 1893, stage 5, plate 1 fig. 50); L, flat surface of an egg showing a common result when first cleavage is latitudinal; the small cell separated from the larger one divides repeatedly before furrows appear in the latter. a. — pale area, d. — dark spot, f. — cleavage furrow, i. — inner membrane, p. — pigment. Scale lines = 1 cm.

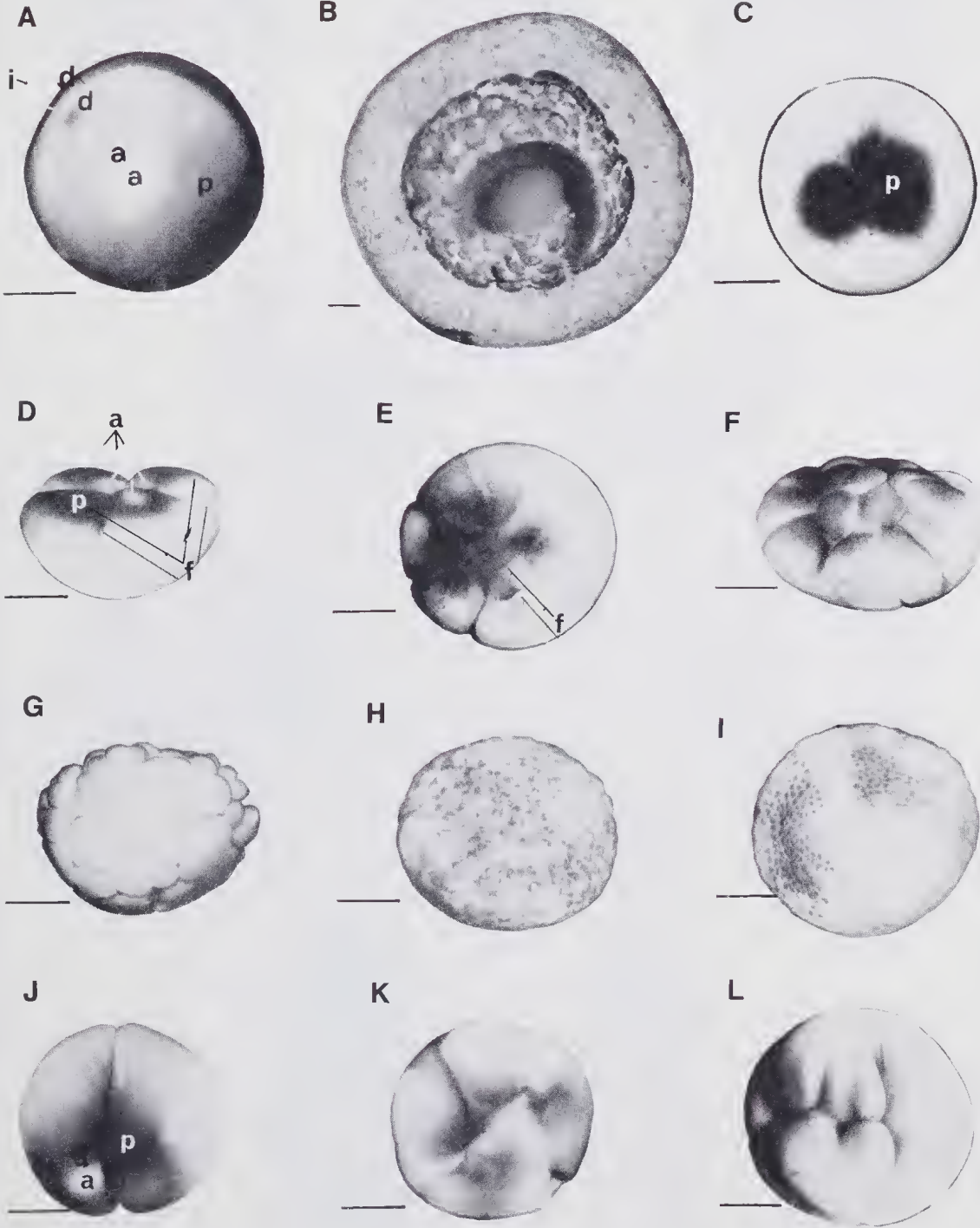


PLATE 2.

FIXED EGGS AND EMBRYOS OF *N. FORSTERI*.

A, B gastrulae, C-E early neurulae F-J, development of the head. A and B, egg within the membranes, C-J after removal from membranes. A, an egg at stage 12 showing early development of the blastopore; pigmented cells are invaginating over the lip, dragging unpigmented pavement cells inwards; B, a gastrula at stage 15 showing the dorsally constricted blastopore with the lateral lips swinging inwards, and the absence of a ventral lip; C, an embryo of stage 17 showing the dorsoventrally orientated blastopore; D, an embryo of stage 18, looking down on the neural plate, which is slightly raised anteriorly and lined with pigment; E, an embryo of stage 22, looking down on the neural folds, showing the transverse crest (sunk inwards) and the primary brain vesicles; F, a lateral view of an embryo of stage 26, showing the otic vesicle and the head growing out from the rounded mass of yolky cells; G, an embryo of stage 30 looking down on the head and posterior part of the ventral surface (the embryo at this stage is almost circular) showing the developing head, presumptive gill region and anus; H, a lateral view of an embryo of stage 32 showing the rounded endoderm, eye, presumptive gill region, pronephros, axis of body, anus and tail bud; I, a ventral view of an embryo of stage 33 showing nares, mouth region (stomodaeum), endoderm, anus and tail bud; J, a dorsal view of an embryo of stage 35 showing tail bud, lengthening body axis, pronephros, presumptive gill region and hindbrain forming under the skin. a. — anus, b. — blastopore, d. — dorsal lip of blastopore, f. — forebrain, g. — presumptive gill region, h. — hindbrain, l. — lateral lips of blastopore, m. — midbrain, n. — nares, n.f. — neural folds, n.p. — neural plate, o. — otic placode, p. — pigment, pn. — pronephros, s. — stomodaeum, t. — transverse crest, t.b. — tail bud. Scale lines = 1 mm.

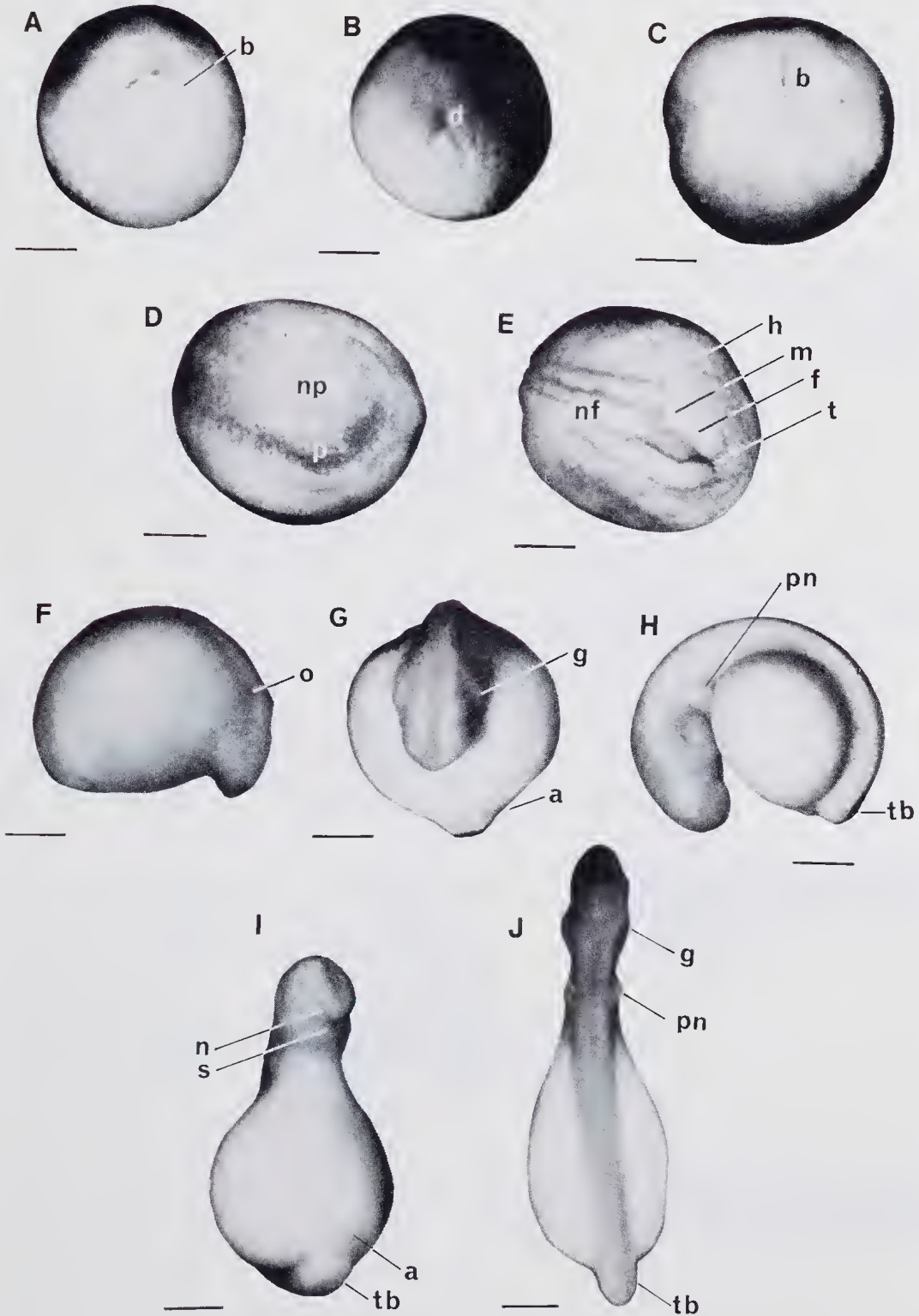


PLATE 3.

EMBRYOS AND JUVENILES OF *N. FORSTERI*

A–C stages prior to hatching, D–H, post-hatching. A, embryo of stage 37 in lateral view showing the platelet arrangement of the endodermal cells, the eye, mouth, primordium of the operculum, somites, melanophores, rectum and the extension of the tail bud, with dorsal and ventral fins; B, lateral view of an embryo of stage 42 showing the extension of the dorsal fin to the back of the head, appearance of the ventral pre-anal fin, the operculum, the eye, the lateral line reaching halfway down the trunk, primordium of the pectoral fin and melanophores covering the whole body; C, animal of stage 44 in lateral view showing the lateral line extending to the tail, the appearance of the supraorbital sensory line of the head, increased growth of all fins, appearance of red chromatophores and development of the operculum and the mouth; D, lateral view of a fish of stage 47 showing remnants of the yolk and traces of the spiral valve in the gut, development of the pectoral fin, mandibular infraorbital, supraorbital and temporal sensory lines and nearly terminal mouth; E, dorsal view of a fish of stage 48 showing the temporal sensory line of the head, pigment in the eye and increase in the melanophores and red chromatophores in the skin; F, fish of stage 49 in lateral view with reduced ventral pre-anal fin and the primordium of the pelvic fin; G, ventral view of a fish of stage 51 showing the developing pectoral fin, sensory line including the oral line broken into a series of pits, skeleton of the pectoral fin, nearly terminal mouth and regression of the pre-anal fin; H, living fish of stage 55 showing pigment spots on the tail and regression of the dorsal fin. d. — dorsal fin, c. — red chromatophores, e. — eye, i.o. — infraorbital sensory line, l. — lateral line, m. — mouth, md. — mandibular sensory line, me. — melanophores, o. — operculum, or. — oral line, p. — platelets of yolky cells, pf. — pectoral fin, pl. — pelvic fin, pvf. — pre-anal ventral fin, r. — rectum, s. — somites, so. — supraorbital sensory line, sp. — spiral valve and yolk remnants, t. — temporal sensory line, v. — ventral fin. Scale lines = 2 mm.

